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BIOLOGICAL, CHEMICAL, AND SILVICULTURAL CONTROL OF WHITE PINE BLISTER RUST

A PROBLEM ANALYSIS

by

Forest Disease Research Staff

Research Work Unit 2303

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INTRODUCTION

Since its introduction to North America in the first decade of this century, the blister rust fungus, *Cronartium ribicola*, has caused tremendous mortality in stands of native white (5-needled) pines in United States and Canada. It has, for the past 40 years, been the object of a sustained control campaign, particularly in commercial stands of eastern white pine (*Pinus strobus*), western white pine (*P. monticola*), and sugar pine (*P. lambertiana*).

The control program, financed largely by the United States government but also by funds from State and private sources, has been conducted primarily by the Department of Agriculture. Since the reorganization of that Department in 1953, control of blister rust has been directed by the U.S. Forest Service. Until recently, control efforts in the western white pine type were largely through removal of the alternate host plants (*Ribes* spp.) growing in association with the pine stands. Although the disease in 30 years spread throughout much of the main commercial ranges of these three major pine species and to other white pine species, it was hoped that many of the commercial stands could be adequately protected by this control method.

THE PROBLEM

The economic limitations of control through ribes eradication has long been recognized, but only recently has its long-range ineffectiveness in western white pine forests been documented (Carlson and Toko, 1968). Therefore, it is apparent that some other control measures must be developed to effectively and economically maintain western white pine as an integral component of managed stands. One control would be through trees resistant to the disease, and development of resistant western white pine is the mission assigned to Research Work Unit FS-INT-2302 (see pages 3, 6, 7, and 33). However, management people will need other means of control to use in conjunction with genetic resistance and, in fact, they need these means long before resistant material could become available. This management need clearly defines our problem: exploration of chemical, biological, and silvicultural means of blister rust control which can be integrated along with resistant stock into effective and economic management of western white pine.

The Problem Base

Forest industries constitute one of the most important segments of the economy in the Inland Empire (western Montana, northern Idaho, and northeastern Washington). Here, western white pine grows on more than 3.6 million acres and occupies a significant place in nine ecological habitats (Daubenmire and Daubenmire, 1968). It has been a most valuable species for forest industry, and its wood is one of the highest quality softwoods available.

Blister rust is prevalent in all western white pine stands in Region One. In 1952, 30 years after introduction of the rust, the disease was estimated[1] to cause annual losses in the Inland Empire of 90 million board feet of white pine sawtimber and the equivalent of 75 million board feet of white pine growing stock. Many remaining stands contain a large percentage of mortally infected trees, many of which might still be saved from premature death if a biological or chemical treatment could be made an effective control measure.

Control of the disease by eradication of the alternate host plant (ribes) commenced about 1925. In time it became apparent that hand or chemical eradication of ribes was impracticable in poorly stocked or isolated white pine stands.

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[1] From data compiled for U.S. Forest Service. Timber resources for America's future. U.S. Dept. Agr. Forest Resource Rpt. 14, 713 pp., illus. 1958.

Timber value of the scattered white pine was insufficient to justify control, and white pine in these areas was abandoned to the rust. Thus, while the area ecologically suited for growing white pine covers 3.6 million acres, that within the blister rust control program of 1952 comprised about 30 percent or 1.15 million acres. The "control area" by then was limited mainly to well stocked stands on the better sites, where the stumpage price differential favoring white pine over mixed species was sufficient to justify the high costs of ribes eradication (Matthews and Hutchison, 1948). The study reported by Carlson and Toko (1968) confirmed earlier decisions to terminate ribes eradication as a control method even on these better sites, and no work of this type has been done since 1966.

In the early 1950's, the Forest Service helped launch and maintain two other programs involving different approaches to control: (1) breeding and developing resistant western white pine trees (FS-INT-2302), and (2) developing a chemical control, chiefly with antibiotics (Pest Control Branch, S&PF, Region 1). Current views agree with the original concept that the two control methods could supplement each other admirably. On the one hand, direct and economic control with a chemical would assure a supply of raw material and thus stabilize the white pine industry for many years to come. While provision of resistant nursery stock cannot replace control by any means in established stands, it will slowly supplement direct control on cutover areas planted successively with the resistant stock as it becomes available.

Experimentation and analysis (Bingham et al., 1969) has established that blister rust resistance in western white pine is a heritable characteristic. Furthermore, estimation of genetic gain in resistance per generation of breeding indicates that, in all likelihood, an early product of the resistance breeding program will satisfy immediate field planting requirements. Altogether, it appears that about 20 years of intensive breeding work (12 still ahead) will be required to put suitably resistant white pine strains into full production. Meanwhile, crossings in arboretums can provide one-sixth of the needed seed.

Personnel of the blister rust control organization in Region One of the Forest Service started an intensive search for a chemical method of blister rust control more than a decade ago. In their search for a suitable fungicide, numerous antibiotic materials were among those tested. In 1956, cycloheximide, an antibiotic provided by the Upjohn Company, showed some promise. Working together, the Pest Control Branch and the Upjohn Company subsequently developed and tested formulations and application techniques for cycloheximide. By 1958, the material was considered worthy of a small scale operational control test. In this same year another antibiotic (Phytoactin) was developed by Pabst Laboratories and field tests were started by Pest Control.

The early tests with these two antibiotic materials on western white pine indicated that the fungus in most rust cankers in treated trees would be killed. However, as the results from the large scale operational program and the Station's experimental areas were followed, it has become increasingly evident that significant inconsistencies occurred (Leaphart and Wicker, 1968). In 1966, Region One did not use Phytoactin and severely curtailed use of cycloheximide by the end of that year for control of blister rust in white pine stands. No antibiotics have been applied operationally from 1967 to the present.

Tests with Phytoactin and cycloheximide have not given satisfactory results on other white pine species, including eastern white pine in the Southeast and in the Lake States, sugar pine in the Pacific Southwest, and white bark pine (Pinus albicaulis) and limber pine (P. flexilis) in Region One. While tests of other chemicals for control of blister rust on all 5-needle pines have likewise been discouraging, the prospects for a chemical control are by no means discouraging. In retrospect, the lessons learned from the "antibiotic epic" have helped formulate and justify the approach used in our current research program outlined here.

Silviculture of western white pine has never had a significant role in blister rust control, except at the regeneration stage. Here, the emphasis was on ecology of Ribes and establishment of full and immediate stocking. Those methods that facilitated the job of ribes eradication and insured desired stocking were favored (Moss and Wellner, 1953). Thus, clearcutting followed by burning and planting came to be the preferred method of regeneration in the white pine type, at least on national forest lands.

While thinning and pruning were advocated as important measures to integrate in rust control programs involving ribes eradication (Moss and Wellner, 1953), they have been rejected as direct control measures for no proven or documented reasons. Their use was advised in conjunction with a ribes eradication program (Moss and Wellner, 1953). Historically, western white pine has been managed for rotations of 100 or more years. Although many acres, e.g., about 4,500 on the St. Joe National Forest, have been pruned for rust control, the idea of using pruning and thinning to curtail rust undoubtedly was not followed up because of the high risk and cost factors associated with protection by such measures over 100-year or longer rotations. Only within the past decade has thinning seemed to have established its place in management to promote growth and thus reduce rotation lengths. Hopefully now, shorter rotations can be economically justified through applied silviculture; surely, they are biologically justified through significantly reduced risk of losses due to pole blight, bark beetles, heart rots, as well as perhaps blister rust.

Relation to Other Problems

Blister rust control is the key to successful white pine management. Without effective curtailment of the rust fungus, white pine will be a minor component of future forest stands of the Inland Empire.

Merchantable white pine on approximately 2.5 million acres must soon be harvested here to avert tremendous losses, for many such stands have suffered 5 to 10 percent annual loss (tree basis) from blister rust in recent years. The rust situation in these stands has been confounded considerably by bark beetle endemic and epidemic activities. If effective biological or chemical control of blister rust was now available for white pine, these merchantable stands might be harvested in a planned, orderly fashion by coordinating such controls with bark beetle control actions. However, assuming a more likely situation of providing such measures, say 5 to 10 years hence, treatment might then still beeconomical depending on the residual pine volume in these stands.

The current policy in management of nonmerchantable (seedling, sapling, and pole) stands discriminates against white pine in cultural operations, such as thinning. Actually, one should assume that any species will have to bear some cost for disease and/or insect control during its rotation. For stands capable of supporting costs of thinning and pruning to improve timber quality and yield, these measures may also prove appropriate for white pine to reduce the hazard from rust. For older stands, provision of biological or chemical means of controlling the rust could insure attainment of merchantable white pine. When a blister rust control measure is assured for western white pine, it likely could then be developed for other white pine species. It also might be used to increase the acreage under management for western white pine, as well as for other commercial white pine species, in the United States and Canada.

Severe widespread damage from blister rust is occurring in white bark and limber pine stands in western United States and Canada. Development of biological or chemical control in these species is urgently needed in Yellowstone and Glacier National Parks and in other rough high elevation country where ribes removal has not been practical or effective. Aside from their esthetic value these pines constitute valuable watershed cover, and on about one million acres in the northern Rocky Mountain states alone they occur in pure stands as the only protective tree cover. More than half of this acreage contains commercial stands of these pines. Considerable mortality from the rust has occurred and as much as 90 percent of the remaining trees in some stands in the northern part of the range of *P. albicaulis* are now mortally infected. Chemical aerial treatment of many of these high elevation forests would be feasible and might be economically justified.

Relation to Station Research

Research on the silviculture of western white pine (FS-INT-1201) has been predicated on suitable control of white pine blister rust. Without control of this disease it would be impossible to grow and manage western white pine stands to merchantable size in the Inland Empire. Forest management personnel indicate future research on this species would use blister rust resistant stock as its principal base for maintaining white pine as a manageable component of future stands and would employ other blister rust control methods, when developed, as supplementary measures. The advent of a successful chemical and/or biologic control method may certainly influence future management. Whether young white pine stands can be treated to minimize lethal infection through silvicultural techniques and, thus, produce a harvestable product under short rotations directly involves Research Work Unit FS-INT-1201 whose role would partially change to active management research for disease control.

As previously indicated, FS-INT-2302 is well along with its task of providing resistant parents for seed orchards. These orchards should be in quantity production by 1985. But whether the white pine populations of stands are relatively susceptible (present stands or those regenerated naturally in the next 20 or more years) or resistant (regenerated through seed from resistant orchards), other control measures tested and developed through the research discussed here can be effectively integrated into a logical control program. If the silvicultural approach incorporating short rotations, increased growth, and reduced target area can be supported by fact, study of those heritable characters (FS-INT-1401) related to growth-rust hazard correlations would have merit to provide characters additive to those within the realm of the disease resistant base for rust hazard reduction. Even within the disease resistance base (FS-INT-2302), greater consideration than currently directed might eventually be given to an overall lower resistant product, to specific inheritable traits that lower the number of infections, and to correlation of field vs. nursery resistance levels etc., should silvicultural control be proven practical and sound.

Several other aspects of the genetics project (FS-INT-2302) have significance to this analysis. The nature of resistance as related to some biochemical constituents of the host has been studied. If the mechanism of resistance should be related to a chemical factor, application of this knowledge might considerably enhance our progress toward selection of a chemical(s) to be used in control of blister rust. Also, the genetics of the rust fungus is relatively unknown. Although it directly concerns breeding white pine for resistance, knowledge of the rust's genetic stability (including its tolerance of systemics) will be useful in formulating plans to control the disease chemically (re: FS-INT-2303-113[2/]).

Questions Needing Answers

Many of the questions posed in the 1965 Problem Analysis by Kimmey and Leaphart remain valid but are not repeated in this revision. New questions since then relate primarily to the biology of the rust organism and the association with its host. Soon after our original analysis, and strongly emphasized today by these new questions, we recognized a significant question inferred in 1965. That question is, "how could we utilize existing and future world literature to direct our ongoing research, to test and confirm hypotheses, and to otherwise facilitate our approach for solving our problem most economically and quickly?" Our answer, narrated later in 2303-104, was to develop an automated information retrieval system. Hopefully, we can soon add this very significant tool to our arsenal for attacking our problem.

We have assumed that the cost of control of blister rust could be borne by western white pine grown for timber. Quite frankly, the question, "how much of a cost burden for disease control can be supported by white pine (or by any other species) and under what conditions?" has not been answered although its relevancy is most pertinent today. For example, if pruning is as profitable a practice for western white pine (Wikstrom and Wellner, 1961) as for eastern white pine (Calvert and Brace, 1969), could young pine stands bear any costs for additional pruning needed to protect them from rust at an acceptable risk level? Pruning at early ages (5 to 10 years) necessary for rust control might incur additional costs, but under what circumstances must these costs be less than those of any other control? Also, rather than question whether chemical control would be financially possible to produce white pine lumber (Peterson and Jewell, 1968), might we rather assume that any effective chemical control would be justified for protection of 5-needle pines used for other purposes?

[2/] Future reference to studies within INT Projects will indicate only project and study numbers.

Perhaps of equal significance is how economics of disease control could influence the direction of our chemical research and testing. At present, our planned course is to assume that such factors as technology of chemical synthesis, translocation and application, the economics of supply and demand of a merchantable forest product, and eventual intensive forest management, will solve the profitability question of any effective chemical or other control method. But while we do not treat the economics of blister rust control in this analysis, it is inevitable that research for control of the disease must, at some future time, be provided a better cost vs. return base than is available today for decision making.

SCOPE

Although this problem might eventually be solved for all white pine species presently affected by the disease, our research will be confined to western white pine. The immediate problem area is confined to the Inland Empire.

PROBLEM COMPONENTS

The 1965 Problem Analysis recognized the antibiotic situation as an integral part of our research. Because our evaluations have shown results in line with decisions made on the use of the antibiotics and because our other research has defined our needs and direction more clearly, a current analysis was in order.

The problem areas have been redefined into three components. Four major changes are notable. The research proposed previously on effects of chemicals has been reassigned to two components, (1) biology of _C. ribicola_ and its hosts, and (2) application of biological information in control measures, with the intent that our quest for basic knowledge about the organism and its relation with its hosts be a clear mandate and that such research would be clearly supportive of the entire program rather than just the chemical phase. Integration of biological information has been identified as the second component of our analysis and will use as its initial base our proposed information retrieval service. A third change involves biological control; where previously only biological agents, primarily fungi, were highlighted, the scope has been broadened to include evaluation of natural canker inactivation (a summary product of all factors). The fourth change adds a new phase of study, i.e., control through silvicultural manipulation of white pine stands.

Each of the closely interrelated components are divided into one or more related areas of study. Research is under way in all components; and all studies (some general) currently visualized to provide clues in answer to our objective are listed and assigned a priority in the program. Some studies, which were shown in the first analysis, have been terminated and are not included in this one.

Consistent with the overall project objective--to provide a biological, chemical, and/or silvicultural control of white pine blister rust, the three components with their immediate objectives are:

1. Biology of *Cronartium ribicola* and Its Hosts.

   A. Cultural parameters. Objectives: To establish the hosts and the rust-host combination in culture; to determine the nutritional and environmental requirements of these tissues; and to define specific factors permitting growth and subsequently physiological study of the rust in pure culture.

   B. Physiology of Disease and Component Organisms.

      Objectives: To determine what biochemical differences exist between the rust and its hosts, to establish which of these metabolic systems are functioning to permit the host-parasite relation, and to learn how the parasite influences the phenolic metabolism, translocation, and respiration of its host.

   C. Epidemiology and Etiology of the Disease. Objective: To establish the reliability of canker growth, seasonal development, and lethality criteria under present conditions and to determine infection patterns in tree crowns in relation to stand density and target area.

   D. Life Cycle. Objective: To delimit the adaptive capacity of *C. ribicola* to substrates *in vivo* and *in vitro*; to ascertain its requirements for sporulation; and to extend our knowledge of its sexual nature and nuclear phenomenon in vegetative and spore stages.

II. Integration of Biological Information. Objective: To provide a means whereby current and recent literature can be queried referent to life and its control and to use that information base in conjunction with our own findings to guide ongoing research, to test theory and concept, or to formulate principles of control of the rust disease.

III. Application of Biological Information In Control Measures.

   A. Chemical. To develop methodology through which we can effectively test chemicals or series of chemicals (natural or synthetic) for control of *C. ribicola*.

B. Biological.

1. Evaluation of Biological Agents. Objective: To assess the potentials of biological agents, primarily fungi, as controls for *C. ribicola*.

2. Evaluation of Natural Inactivation[3]. Objective: To assess the significance, including related ecological factors, of natural inactivation of blister rust infections and to test remote sensing and other techniques for disease evaluation in the field.

C. Silvicultural. Objective: To test those silvicultural procedures that promote growth and reduce target areas of white pine for effective control of the blister rust disease.

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[3] Referred to in this analysis as encompassing all ecological or physiological causes, which are not the result of man's direct activities, e.g., biological agents, host resistance, branch death, etc., that individually or collectively induce inactivation of infections.

RESEARCH PROGRAM BY PROBLEM COMPONENTS

A limited review of the literature, pertinent research to date, and proposed studies with their objectives are presented here for the areas of study within each component. This information provides the background for the section that later follows on priority within the research program.

I. Biology of *C. ribicola* and its hosts

Control of white pine blister rust is faced with a perplexing problem, the obligate nature of the pathogen. This problem appears particularly significant for control with chemicals in view of the lack of success and cost of the search for therapeutic chemicals for organisms which are pathogenic to plants but are not obligately parasitic. Our approach to control is further complicated by a lack of knowledge concerning the life cycle of the rust fungus and the biology and biochemistry of the fungus, its hosts, and the host-parasite combination. However, since the environmental requirements of obligate plant rusts are highly exacting, they appear highly vulnerable to control through their artificial disruption. Thus, what may be an obstacle in the beginning can become an advantage if these requirements can be defined for this rust fungus both within and outside its hosts' tissues.

Cultural Parameters

Paramount among the problems which must be solved, at least to some degree, before an intelligent and economical search for chemical controls can be initiated is the definition of the nutritional and adaptational limits of the rust either in pure culture or in cultures of its hosts' tissues. Provision of such cultures gives us a means of assessing the control potential of candidate chemicals in a controlled environment and a tool for direct study of rust physiology. It is also reasonable to assume that comparison of the nutritional requirements of healthy and infected host tissue will provide insight to the physiology of the rust and the host-parasite system.

Literature Review.

The obligate parasitism of plant rust fungi has traditionally been regarded as absolute. However, in light of the sporadic reports which have appeared since 1901 (Yarwood, 1956), use of the term obligate parasite, when applied to fungi, may soon be restricted to "under conditions prevailing in nature."

Obligate parasitism has been commonly regarded as having evolved from obligate saprophytism through facultative saprophytism and possibly symbiosis (Yarwood, 1956). If this evolutionary scheme is accepted, it follows that some isolates of these fungi should retain at least part of the capability of their predecessors to grow on a less restrictive substrate. Such an assumption, of course, depends on the frequency of the loss of the genetic material required for this capability during the time since it was required for the survival of the organism. Loss of genetic material (deletion) does not occur frequently; and since several obligate parasites have been cultured, the assumption therefore appears valid. Readaptation of obligately parasitic fungi to nonliving substrata, either by induction of an innate but unused capability or through selection of reverse mutants is plausible. Such a hypothesis has been advanced by Williams et al. (1967), and by Hotson and Cutter (1951) to explain, at least partially, their success in culturing *Puccinia* and *Gymnosporangium*.

Development of a technique for the *in vitro* culture of plant tissues, Gautheret (1942) and White (1943), introduced the possibility of culturing tissue infected with an obligate parasite, thereby pre-conditioning it for a saprophytic growth habit. Morel (1948), however, was unable to infect healthy host callus tissue with either urediospores or basidiospores of *Puccinia antirrhini*, or *P. malvacearum*, or aeciospores of *Gymnosporangium clavariaeforme*. Hotson and Cutter (1951), Cutter (1951, 1959), and Hotson (1953) were notably successful at growing an isolate of *Gymnosporangium juniperi-virginianae* in tissue cultures of its coniferous host with limited subsequent movement of the rust onto the medium and saprophytic growth. In addition, Hotson (1953) successfully inoculated healthy calluses in culture with this fungus. Turel and Ledingham (1957), however, failed to establish the fungus on artificial media but were successful in growing *Melampsora lini* on cultured cotyledons of flax. Cutter (1952, 1960) was also successful in culturing *Uromyces caladii* and *U. ari-briphylis* on host tissue cultures. Maheshware et al. (1967) were unable to obtain growth of *Puccinia helianthi* or *G. juniperi-virginianae* in proliferating callus tissues of their respective hosts. Bauch and Simon (1957) obtained mycelial growth and production of teliospores of *Puccinia tartarica* on tissue cultures of rust infected wild lettuce. Rosetti and Morel (1958) grew *Puccinia minutissima*, and *Gymnosporangium juniperi-virginianae* on tissue cultures of their respective hosts. Walkinshaw et al. (1965) cultured slash pine cambial tissue infected with *Cronartium fusiforme*. Nozzolillo and Craigie (1960) grew *Puccinia helianthi* on tissue cultures of its host.

Koenigs (1968) observed invasion of the rust fungus, *Cronartium ribicola*, into callus cells of western white pine cambium, and Harvey (1967a, 1967b) reported establishment of infected white pine tissue in axenic culture and formation of aerial mycelium. Subsequently, monokarytic vegetative mycelium from the blister rust fungus has demonstrated the capability of reinfecting callus tissue (Harvey and Woo, 1969; Harvey and Grasham, 1969), the capability of surviving (separated entirely from host tissues) for a limited time on artificial media (Harvey and Grasham, 1970b), and of surviving and growing in the absence of direct contact with host tissue on artificial membranes (Harvey and Grasham, 1970a).

Cultures of vegetative mycelia from a rust fungus have also been established directly from spores, although Yarwood (1956) and Shaw (1964) had no success in culturing rust urediospores. Williams et al. (1966, 1967) have reported significant success in culturing *Puccinia graminis* f. sp. *tritici* from urediospores. Mycelia from urediospores successfully reinfected wheat.

The bunt fungi, long considered obligate parasites by many phytopathologists, have also succumbed to artificial culture. Fischer and Holton (1957) felt that these fungi should not be considered obligate parasites because many species have been reported to produce teliospores in sterile culture. All such reports, with two exceptions have, however, dealt with the mononucleate, non-pathogenic, stage of the bunt fungi. These two exceptions, Kendrick (1957), Trione (1963) reported culture of the pathogenic stage.

The establishment of axenic cultures of obligate plant pathogenic fungi, with and without their hosts, for study under controlled conditions has, therefore, been demonstrated, thus, proving that the obligate nature of some parasites is not absolute.

Investigational Approach

To date considerable progress has been made in defining the cultural parameters of the host, the parasite, and the combination. It is therefore reasonable to assume the general approach outlined below will continue to provide answers in this problem area.

Phase I. Methods for culturing host tissues must be developed and are considered a necessary first step in establishing clones of host and pathogen.

Phase II. Methods must be developed to grow tissue cultures in a liquid substrate. This substrate can then be used as a supplement to culture media. Callus diffusates avoid complications caused by wound reaction metabolites. Such metabolites are highly toxic to microorganisms.

Phase III. Analysis of the major nitrogen and carbon sources contained in healthy callus cultures will be used to modify the culture medium so it resembles, as closely as possible, the normal fungus substrate, i.e., host tissue.

Phase IV. Tissue culture methods must be developed to introduce the rust fungus into actively growing, healthy host callus cultures.

Phase V. Relatively large numbers of infected tissue cultures, representing diverse genetic material, must be maintained, thus, providing maximum tissue parameters for obtaining culturable mutants. In addition these clones provide an extended period of time for culturable mutants to readapt to non-living substrates.

Phase VI. Fungus mycelia from selected cultures demonstrating extensive capability of growing on or adjacent to callus tissue can be transplanted to: (1) media formerly occupied by healthy callus; (2) media containing embedded healthy callus covered by artificial membranes; and (3) chemically defined media.

Phase VII. Analysis of media supporting vegetative growth of the rust fungus can be made and the nutritional capabilities and limitations defined.

Phase VIII. Comparisons can be made between the nutritional limitations of various isolates of the rust fungus with differing pathogenic and morphologic characteristics. Such information should provide some insight to the reasons behind the (substrate) specificity of this organism and its ability to adapt to environmental change.

Progress to Date

The fulfillment of the objectives of Phases I and II are nearing completion. Work now completed under the auspices of study 2303-90 has provided the following information:

1. Methods to culture cambium, needle, and embryo meristematic pine tissue on semi-solid medium.

2. Methods for culturing pine stem cambium tissue in a liquid medium.

3. Methods to culture ribes stem and petiol tissue on semi-solid media.

4. Methods to culture stem tissue from 12 additional conifer species.

Although the complete nutritional and physical environmental requirements have not been completely worked out, sufficient information has been obtained to move on to the next phases.

Partial fulfillment of the objectives of Phases IV, V, and VI have been realized under study 2303-93. The following significant progress has been made in this study area:

1. Methods have been developed for infecting healthy pine callus.

2. Methods have been developed for propagating infected callus.

3. Methods have been developed for propagating vegetative clones of the rust fungus.

4. Methods have been developed for maintaining a culture bank of rust tissue.

5. Have attained invasion of an artificial medium by fungus mycelium.

6. Have survival of the rust fungus on an artificial medium after removing the living infected host tissue.

7. Have vegetative growth of the rust fungus in the absence of contact with its host through separation of the host and fungus by cellophane membranes.

8. Have five suspected rust cultures on artificial media.

A small amount of pilot work has been done on Phase III and the information has been incorporated in the makeup of test media. However, major effort here has been delayed for further confirmation that vegetative growth has been attained on analyzable non-living substrate. Such confirmation appears imminent. (See items 5, 6, and 7 above achieved through Phases IV, V, and VI.)

Major effort on Phase VIII must await the completion of Phase VII and reasonable confirmation that identifiable cultural variates and substrate adaptation are innate characteristics of the rust organism (reference also to Study D 1-a, page 34).

Future Study

At present, the remaining primary obstacles to defining the cultural parameters of the blister rust fungus (nutritional, biological, and physical requirements for survival) have not been accomplished.

1. Vegetative growth of confirmed fungus isolates separated from living host tissue.

2. Identification of the factors responsible for this growth and the cardinal levels required.

3. Establishment of the dikaryotic mycelium in *Ribes* tissue cultures.

4. Establishment of single spore lines of rust.

5. Utilization of the various spore stages for study of the histochemistry of the infection processes and as a means of establishing rust in tissue and axenic culture. (FS-INT-2302 is working out this area with sporidia on pine leaves.)

In addition, much routine work has been and must be passed over in favor of the more pressing barriers to progress. However when possible, this work should be completed in order to have all parameters for growth of the rust fungus fully defined. Study areas requiring further effort are:

1. Full definition of carbon sources, organic nitrogen sources, and vitamin requirements of healthy and infected pine tissue cultures.

2. Full definition of mineral requirements for healthy and infected pine tissue cultures.

3. Full definition of light, temperature, and atmospheric requirements of healthy and infected pine tissue cultures and their effect on fungus growth habits.

Problem Area Outline

Current and proposed studies are summarized here with their objectives according to organism. Table 1 is based on the following format.

A. Cultural Parameters

    1. Host

   a. *2303-90*. Nutritional and environmental requirements of axenicly cultured western white pine tissue. Objective: To investigate the nutritional and environmental requirements of tissue explants of *Pinus monticola* cultured *in vitro*.

   b. *2303-123*. Nutritional and environmental requirements of axenicly cultured *Ribes* tissues. Objective: To modify cultural substrates used for cambial tissue of *Ribes* so as to effectively grow other tissues, particularly leaf components, in culture as a tool for establishing the binucleate phase of *C. ribicola* in tissue cultures.

2. Host-parasite combinations

   a. *2303-93*. Feasibility of establishing white pine tissue explants and/or subcultures, infected with *Cronartium ribicola*, in axenic culture. Objective: To establish infected white pine tissue in axenic culture for use both as a tool to investigate the host-parasite relations and as a bridge to aid in establishing the rust fungus in axenic culture.

   b. *2303-126*. Nutritional and environmental requirements of white pine tissue cultures infected with *C. ribicola*. Objective: To study the effect of pathogenesis by *C. ribicola* on the normal nutritional and environmental requirements of cultured white pine tissue.

   c. *2303-*______. Supplement to 2303-93 specifically for tissues of *Ribes*. Objective: To establish the binucleate phase of *C. ribicola* in tissue cultures of the alternate host.

3. Parasite.

   a. *2303-98*. Establishment of *C. ribicola* in axenic culture. Objective: To develop a method for growing *C. ribicola* on artificial media (from its spore stages) and to assess the nutritional and environmental requirements for its growth.

b. 2303-124. Pure culture of *C. ribicola* from its vegetative stages. Objective: To establish the vegetative stages of *C. ribicola* on an artificial substrate.

c. 2303-127. Isolation and identification of the specific factors supplied by host tissues that are required for the independent survival and growth of *C. ribicola*. Objective: To determine the factors responsible for survival and growth of *C. ribicola* on complex organic substrates.

d. 2303-____. Nutritional and environmental requirements of *C. ribicola* in pure culture. Objective: To fully define the spectrum of nutritional and environmental requirements of *C. ribicola* on artificial substrates.

Physiology of Disease and Component Organisms

In nature the susceptible pine and ribes hosts provide an environment favoring infection by Cronartium ribicola through provision of factors that satisfy the obligatory nature of the fungus. A biochemically oriented approach to identifying these factors involves analysis followed by synthesis. First, one isolates the factors and studies their properties. Second, from this information plus observations on the intact system one devises a plausible model of how the complete system works.

Physiological similarities between organisms do not lend themselves readily to involvement in biochemical control, therefore, this phase of research emphasizes an approach directed toward identifying physiological differences between host and pathogen and physiological processes under direct or remote regulation by each participant or their interaction.

Current State of Knowledge

Analysis of cell wall constituents with histochemical techniques showed the predominant polysaccharide of fungal walls to be chitin while both hosts demonstrated a prominence of cellulose and the absence of chitin (Martin, 1967). The sugar precursor to chitin synthesis is fructose, a readily available molecule from the cleavage of host sucrose. Cleavage of sucrose also provides the glucose necessary for glycolysis or pentose phosphate shunt and probably provides the major source of metabolic energy for both organisms. Although the regulation of these pathways and the translocation of precursors and products are important to understanding the host-parasite interaction, continued study of these processes is unwarranted now since it appears unlikely that introduced metabolites could effectively block chitin synthesis. Furthermore, study of chitin metabolism involves instrumentation not currently available.

Pectin and calcium are frequently associated with cell walls and are in high concentration in the cementing material between host cells. In blister rust infested tissue pectin was not detectable but occurred between cells in tissue void of rust mycelium (Martin, 1967). Apparently the degradation of cell wall and intercellular cement with cellulases and pectinases is involved in pathogenesis in allowing ramification between cells and penetration by haustoria (Tani, 1967). Pectinases and cellulases are mixtures of enzymes specific in their action, a product of a specific reaction becomes the substrate for the next degradation step (Bateman and Millar, 1966; Wood, 1960). The absence or inactivation of a degradative enzyme in the sequence would affect the virulence of the pathogen, or, conversely, be a host-resistance factor. Appraisal of blister rust infected callus (2303-109) should demonstrate the quality and quantity of involved pectic enzymes as well as the toxic influence of indigenous phenolic substances on the activity of these enzymes.

Phenolics and sugars available in white pine are known to be type precursors for glycoside synthesis, a mechanism proposed as a detoxifying reaction for phenolics (Pridham, 1960; Flood and Kirkham, 1960). Glycosylation of phenolics could explain the needle spot coloration associated with infection (Harbone, 1964), an association under study in FS-INT-2302. A single sugar moiety addition to the proper phenolic by a non-specific enzyme may result in a yellow pigment while the specific addition of more sugar would produce a di- or tri-glycoside, a red pigment. Because of the specificity of enzyme for substrate the glycosylation of phenolics, i.e., pigment formation, is a feasible approach to separating physiologic strains of the pathogen as well as a working hypothesis in pathogen-host compatibility (Hadwiger and Schwochau, 1969).

The pathogen sustains itself through intimate contact with host cells but not to the extent that the host cells die rapidly, a condition which would jeopardize the existence of the obligate organism. An explanation for the balance between host and parasite could be found in the accumulation of host metabolites at the infection center (Durbin, 1967). Translocation of the metabolites from other parts of the host toward the pathogen in effect dilutes the fatalistic influence of infection through provision of a larger reservoir of nutrient to the pathogen and the adjacent host cells. In general, the circulation pattern for each nutrient is unique for that nutrient and is influenced by the physiological state of the host, the development of the tissue or organ, and by the environment (Will, 1957). The influence of infection sites upon these patterns of movement is an important consideration in assessing the magnitude of altered host physiology. Available evidence from our laboratory suggests that each infection site behaves as a metabolic sink for some inorganic solutes and that it competes with the normal ones of the host (Martin, 1968). The magnitude of the sink parallels the pathogen's growth. The greater the frequency of infection, the greater is the effect on the host; but low levels of infection do have a disproportionately large influence on the white pine host.

Evaluation of the protein spectrum in white pine tissues (2303-101) has been hindered by extraction difficulties. Bark tissues contain a large number of acids, phenolics, etc., which tend to mask, precipitate, or alter protein (Pridham, 1960; Anderson, 1968; Loomis and Battaile, 1966; Hulme and Jones, 1963; Williams, 1963). To date a relatively small percentage of the total nitrogen has been identified as a protein component. The job ahead is to increase the efficiency of recovery of protein so that the enzyme component can be qualified and quantified.

Energy relations of diseased plants suggest a practical application of remote sensing to disease evaluation. If heat release at an infection site can be related to the viability of the pathogen at the site, remote sensing techniques based on this relationship could greatly facilitate canker evaluation. Even their application at the level of specific tissue can be envisioned. For a number of plants infected with parasitic organisms, higher temperature at infected areas was recorded than in controls (Allen, 1953). Yarwood (1953) measured the heat of respiration in diseased and injured leaves. With 14 out of 15 diseases studied, diseased leaves reached a higher temperature than healthy leaves. Temperature difference ranged from several tenths of a degree centigrade to nearly 1.5°C. He also listed the principal factors affecting leaf temperature. He stated that radiation was thought to be one of the most important factors. In summary Yarwood stated that his calculations indicate that most of the energy of respiration was released as heat under the conditions of his tests. Shaw (1963) recognized a need for measurement of heat production in relation to respiration and demonstrated an increase in respiration rate accompanied by an increase in temperature of diseased tissue. He also noted other physiological changes in diseased tissue.

Translocation pathways in trees have been studied for many years with little success in clarifying the complete picture of what moves where, how, and when (Kramer and Kozlowski, 1960). However, one generally concludes that one element or compound will move by different pathways and by different mechanisms in the tree than will a second compound. The influence of diseases, particularly a canker disease like blister rust, on translocation in a tree merely complicates evaluation of the unknown. Nevertheless, whatever insight one can gain of translocation pathways in infected trees would facilitate our research on systemic chemotherapeutants.

If one can assume that the pattern of movement of dye reflects the usual pattern for movement of elements and compounds, then study of its distribution pattern through a tree could reflect a general pattern for many materials soluble in water. Several workers, notably Greenidge (1955a, 1955b, 1957), Mitchell (1967), and Vité (1959) have studied water movement through translocation of dyes. More recently, Hopkins (unpublished) has used dyes to study translocation in lodgepole pine stems bearing Atropellis cankers. Preliminary evidence suggests that the Atropellis fungus is blocked from the normal pathway of water movement by resin infiltration of tissues in advance of the canker perimeters. Our study of translocation with dyes in infected white pine (2303-107) suggests a differential movement of dye in xylem and phloem healthy tissues as well as between healthy and diseased tissues.

Proposed Research

In a host-parasite complex, like blister rust and pine, many metabolic systems and pathways exist and warrant study. Some, which probably function in the pine-rust system, merit little study since they offer no opportunity to be blocked by chemical alterations without damage to the host. Some, like chitin metabolism, need only be identified to establish their role in the complex. However, others appear to play the dominant role in the pathogenesis peculiar to the rust-pine relation and, thus, are highlighted as initial target systems.

Extraction, identification, and kinetics of proteins are especially warranted. Either qualitative or quantitative differences in the protein spectra of healthy and infected tissues could be indicative of the changed enzymatic conditions requisite for the sustenance of the rust-pine relation. For example, pectic production is but one of several properties which determine the pathogenicity of a pathogen. *C. ribicola* either has this property or can induce the host to produce appropriate pectic enzymes necessary for ramification. A determination of the enzymes involved will yield information referent to the extent of insoluble pectin degradation and the quality of soluble subunits available for further metabolism by *C. ribicola* or by secondary inhabitants of blister rust tissue, specifically *T. maxima*.

Also, altered permeability of cellular membranes could jeopardize the function of an enzyme through accumulation of products or limiting substrate and/or cofactors. If the enzyme(s) involved are a vital link early in the disease process then elaboration of its function and cellular location could lead to formulation of an efficient and complete eradication of the pathogenic organism and its effects on the host. A rate constant as determined on an isolated enzymatic reaction or sequence of reactions under known conditions is a standard for comparison of imposed treatments. The greater the deviation from the standard the more likely it is that selected chemicals have promise for further testing *in vivo* in the callus chemical testing scheme.

Carbohydrate utilization, function, and translocation are emphasized primarily because of results of studies of the biochemistry of resistance. For example, *Pinus* has numerous phenolics in its bark tissue, but little is known of their role in metabolism of the host or fungus. The interaction with enzymes in a manner deleterious to the fungus as well as advantageous to the fungus is possible. The detoxification of phenolics or the establishment of a favorable pH for fungus growth may be a feature of susceptible white pine.

The evidence for the suppression of enzyme activity by phenols appears frequently in literature (Suzuki, 1965). Conifers in general, in this instance *Pinus*, have numerous phenolic species present in adequate concentration to inactivate pectinases and cellulases. Cell compartmentalization and/or uniting a phenolic species with another molecule could render the phenolic moiety harmless to enzymes. Hypothetically, *Cronartium ribicola* could have enzymes or could induce synthesis of enzymes in pine that attach glucose molecules to phenolic species. In doing so, the phenolic compounds are no longer toxic to *C. ribicola* because in this state they cannot inactivate pectinases and cellulases or other enzymes involved in pathogenesis.

The synthesis of glycosides could be a convenient mechanism for detoxifying phenolic molecules. The resultant pigments could function as regulators of sporulation as well as substrates for metabolism. After being translocated as an intact glycoside, or as the components, the molecule could provide to the fungus a sugar metabolite and an aglycone moiety acceptable for further metabolism. Elucidation of enzyme type and site of origin involved in the fungus ramification and establishment process would aid in formulating attack points and mechanisms for their inactivation.

Our interest in mineral extraction, function, and kinetics centers not only on mineral involvement in the physiological processes of the host and host-parasite complex (Neilands and Stumpf; 1958, Durbin, 1967), but how these minerals can alter or be altered by chemicals introduced into the tree's translocation system. The preliminary evidence that only one stem infection can alter the "normal" accumulation patterns in host tissue quite removed from the infection site is perhaps indicative of the importance of study in this field.

Infection centers accumulate metabolites in direct competition with host metabolite centers. The accumulation of metabolites is vital to the existence of the fungus because without it the adjacent host cells would be depleted of vital nutrients to the point of cell death, consequently the death of the obligate fungus. Inorganic solutes detrimental to fungus metabolism must be translocated away from the site or be insensitive to the influence of mobility factors involved in metabolite accumulation. Depletion of metabolites necessary for the development of host tissue could adversely affect growth and reproduction of the host.

The extent to which the physiology of pine is altered by rust infection prior to measurable influences on growth is unknown, aside from the effects of girdling cankers. The more significant aspect is the effect of the rust on translocation patterns of water and solutes and on energy relations. If released energy can be measured, it could provide us with a means of interpreting viability of cankers by instruments. The effect on water relations has a direct or indirect influence on translocation of introduced chemicals.

Research in water relations, photosynthesis, carbohydrate translocation, nitrogen metabolism, nucleic acid metabolism and toxin metabolism await, like research needs in other problem areas, an increase in professional and technical staff coordinated with additional facilities and equipment. For example, study at the cellular or tissue level would not be complete without employing radiotracer techniques.

Problem Area Outline

Current and proposed studies with their objectives are briefed as follows:

B. Physiology of Disease and Component Organisms

1. Identification of systems

a. Protein extraction, function, and kinetics

(1) 2301-101. Blister rust physiology I. Extraction of protein from white pine bark. Objective: To develop an extraction procedure for the proteins of western white pine bark, which will provide:

1) Proteins in native state
2) High protein yield
3) An extract representative of: (a) the protein spectrum of white pine bark, (b) a proportionate concentration of each member of that spectrum.

(2) 2303-109. Pectic enzymes associated with blister rust. Objective: To determine the quality and quantity of pectic enzymes involved in the ramification of *Cronartium ribicola* in western white pine callus tissue.

(3) 2303- . Identification of enzyme function in blister rust disease and role in sustaining *C. ribicola*. Objective: To determine the subcellular compartment(s) containing the enzyme(s) of interest and to determine the enzyme's contribution to establishing and maintaining the disease.

(4) 2303- . Kinetics of enzyme reactions involved in blister rust. Objective: To determine the rate constant for the enzyme(s) of interest and the sensitivity of the enzyme(s) to manipulation by exogenously supplied selected chemicals.

b. Carbohydrate utilization, function, and translocation

(1) 2303-128. The influence of blister rust (*C. ribicola*) on the seasonal distribution of carbohydrates in needle and bark tissue of western white pine. Objective: To determine the effects, both qualitative and quantitative, of blister rust on the seasonal distribution of carbohydrates within needle and bark tissue of *Pinus monticola*.

(2) 2303- . Role of phenolics in fungus metabolism. Objective: To determine the influence of known, indigenous phenolics in restricting or enhancing fungus metabolism and growth.

(3) 2303- . Flavonoids--their synthesis and involvement in host and fungus metabolism. Objective: To determine the flavonoids present at different stages of infection, colonization, and sporulation. To identify the precursors for flavonoid synthesis and the products of degradation.

c. Mineral extraction, function, and kinetics.

(1) 2303-___. Translocation of inorganic solutes as influenced by blister rust. Objective: To determine the influence of *C. ribicola* in establishing metabolite sinks at the infection site and the magnitude of infection influence in host metabolites distal to the infection site.

2. Translocation and accumulation

a. 2303-107. Translocation of dye in western white pine infected with blister rust. Objective: To trace the distribution of dye materials through rust infected portions of western white pine stems and branches and to compare the patterns by type and condition of cankers according to class of tree.

3. Energy relations

a. 2303-119. Temperature measurement of white pine tissues: Healthy and infected with *C. ribicola* Fisch. Objective: Using callus, seedling, and tree materials, determine the temperature of diseased and healthy tissues under controlled and random regimes.

b. 2303-___. (Supplemental to 2302-121). Breaking dormancy in western white pine. Objective: Using seedlings from known crosses, determine the relative quantity of succinate dehydrogenase in cells of the meristem at the end of various chilling periods and correlate the capacity for resuming normal growth and enzyme potential and location.

Epidemiology and Etiology of the Disease

Perhaps the most studied of the problem areas in this analysis has been that of epidemiology of blister rust. However, the previous emphasis has been oriented to ribes eradication. The information from our study of it will be directed first at improving criteria for silvicultural treatments of white pine for rust control and second at improving criteria used in canker evaluation.

Current State of Knowledge

How sound is the biological basis for proposing tests of the silvicultural treatments, i.e., pruning and thinning, as control measures of blister rust?

Etiology of the disease reveals these observations about the rust organism in relation to its host.

1. Under natural conditions, infection probably occurs only through needles, and the highest proportion of bark infections result from infection of needles at the end of their second growing season (Chapman, 1934).

2. The time period from infection in the needle to appearance of the organism in the bark phloem has not been fully studied. While cankers do appear by May on twigs at the base of needles infected the previous fall, the percentage of needle infection producing bark infections within one year may be relatively low (Slipp, 1949). Our data show that needle retention on trees is greater in the lower crown, 3-4 years, than in the upper crown, 2-3 years.

3. Rate of canker growth in branches is variable; but for our canker population has averaged 3.7 cm. of annual growth toward the bole for lethal and 3.9 cm. for non-lethal cankers for a 2-year period from 1966 to 1968 (2303-91). This rate of growth is not affected by relative tree growth rate, i.e., growth of branch cankers for trees that grew 3.5 feet or more in height in the last two years (1967 and 1968) is not significantly different from trees growing between 1.5 and 2.0 feet; 3.8 vs. 3.7 cm. per year, respectively, for lethal cankers.

4. Nearly all infections in trees 10 years and younger are lethal infections and as tree age increases the proportion of lethal cankers in the total canker population decreases (based on our data approximately 36 percent at 40 years). Data from the 1966-67 R-1 surveys (unpublished) show 98 percent of the infections are lethal in trees at 6 to 10 years of

age, 73 percent at 16-20 years, 41 percent at 26-30, and 34 percent at 36-40. Averaged over all trees in our sample, though recognizing the confounding effect of age-growth relations, the faster growing trees have a lower proportion of lethal cankers than the slower growing trees.

5. Whether a branch is or is not flagged is usually essential for judging lethality of cankers; for, until the branch is flagged, any canker is a lethal one. However, current criteria (mostly from Slipp's unpublished data) used to judge canker lethality involve, primarily, diameters and lengths of annual branch growth segments and distance from infection to the bole, e.g., the smaller the branch, the shorter must be the length of the growth segment for a canker to successfully cross it. Annual terminal branch increments on open grown trees for the first two growth segments from the bole (from data in FS-INT-1201) average greater than cankers can successfully cross according to current criteria. Data from our canker sample (2303-91) suggest that current criteria is sound but tends to under estimate the chances for a canker to be lethal.

6. Data on natural inactivation (2303-91) show approximately 24 percent of the lethal cankers became inactive before they reached the bole, and 44 percent of the non-lethal cankers were inactive in 1969. While the permanency of inactivation is indeterminate now, the longer a canker remains on a branch its chances for natural inactivation increase.

Epidemiology of the disease also influences the biological soundness of silvicultural control. The following observations are pertinent.

a. Height of uppermost infections in trees varies with tree height, and thus tree age; and on the basis of our lethally infected tree population (2303-91), about 75 percent of the lethal infections occur in the lower one-third of the crown. Our data also suggest that about 70 percent of the lethal infections occurring in the first 20-30 years of a tree's life would be in the 17-foot, prunable zone.

b. The data by Carlson and Toko (1968), which show an annual increase of about 3 percent of the stems infected per year in young stands, would suggest that ribes populations do not materially alter this infection pattern. Since more than one-half of the stands between 15 and 25 years of age have more than 60 percent of the white pine trees infested (90 percent maximum), one can expect extreme variation among areas. On the other hand, the relatively low infection intensity per tree, averaging less than four infections per tree up to 25 years, coupled with the high proportion of trees infested suggests rather uniform infection within stands.

c. The reason for the great variation in infection intensities among stands warrants close examination. However, a clue is provided from the tabulation, page 31, to suggest that a sufficient number of stems in many young stands could be brought to treatable size with or without ribes removal. Regardless of the number of ribes removed, the number of times the stand was worked, the time interval between workings, or whether the stand was worked prior to, during, or after stand establishment, the within treatment variation hinders assigning much significance to the effect that our manipulation of the ribes population has had on rust infection in young stands.

d. Microenvironment at a tree is more favorable on the average for infection in the lower portion of the crown, in closed canopies, and below the general "brush" vegetation level. Whether "wave" years would provide relatively uniform conditions for infection throughout the tree is unknown; but, if wave years have existed in stands in our sample, we have no evidence yet that the infection pattern in (a) above has been materially altered. Examination of unpublished data on 26 stands (up to 20 years old) from the R-1 surveys reveals no regionwide wave years, but some years (particularly 1962 and 1963) were more often represented by significantly higher numbers of infections than were other years, with considerable variation among stands.

Proposed Research

Much of our current information about canker characteristics comes from our study of status and trend of natural inactivation (2303-91). We propose to reassign some aspects from that study into identifiable and, possibly, short term studies. Although some, such as growth rate, have been studied in the past, we believe that current conditions may be different and, if so, will reflect different canker development patterns. Furthermore, while results of proposed study might provide the ultimate criteria for silvicultural treatments, we propose to install treatments as quickly as possible based on existing data (see second paragraph, page 67), and subsequently refine those criteria as results warrant. In some cases, we shall assess the influence of silvicultural treatment on infection criteria as well as on canker development. Such study will be especially pertinent in properly timing proposed silvical treatments in the future.

No. of Stands	Percent of Stems Infected				Age of Stand		Total No. Ribes Removed		No. Workings		Years between 1st and last workings	
	Total		Lethal									
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
No.	Percent				Years		No.		No.		No.	
First Ribes Working Conducted 1 to 5 Years Prior to Stand Establishment												
22	34	4-55	31	4-54	11.8	4-21	35	0-360	2.9	1-5	8.1	0-17
Same Year of Stand Establishment												
17	34	8-89	31	8-84	11.0	5-19	28	1-116	2.7	1-4	5.2	0-11
1 to 2 Years After Stand Establishment												
5	31	4-88	30	4-86	9.8	5-19	33	2-75	2.4	2-4	4.0	2-7
3 to 4 Years After Stand Establishment												
4	40	14-88	38	14-85	14.8	9-21	33	3-75	2.8	2-4	6.0	3-9
5 or More Years After Stand Establishment												
5	42	10-57	36	10-50	13.0	6-17	24	0-113	1.4	1-2	1.0	0-3

(All figures are compiled from unpublished data from R-1 blister rust incidence surveys, 1966)

Problem Area Outline

Proposed studies with their objectives are outlined for this problem area as follows:

C. Epidemiology and Etiology of the Disease

1. Canker characteristics

   a. 2303-___. Canker growth rates. Objective: To determine the annual growth rates of natural cankers.

   b. 2303-___. Seasonal and annual development and expression of canker activity. Objective: To determine the frequency and magnitude of changes in activity as expressed by macroscopic symptoms by making frequent examinations of selected cankers used in 2303-91 on a year-round basis.

   c. 2303-___. Lethality of branch cankers. Objective: Using cankers used in 2303-91, and others, determine the effects of annual branch segment length and diameter and distance from bole of cankers on the probability of branch cankers reaching the bole.

2. Infection patterns

   a. 2303-118. Distribution of blister rust infection in western white pine tree crowns. Objectives: (1) Establish the pattern of rust intensification in tree crowns over time, as influenced by stand characteristics, especially those induced by silvicultural treatments; (2) evaluate target areas (e.g., stem foliage, short shoots, branch type) as rust hazards, and (3) clarify the influence of favorable infection years (wave years) on intensification patterns. (To be supplemented by data from 2303-91.)

   b. 2303-___. Effects of microenvironment on infection pattern and intensity. Objective: To define factors of the microenvironment that influence infection pattern and intensity. (See also III-B-2b.)

Life Cycle

As was previously pointed out in the relationship of our research to that on "developing white pine resistant to blister rust," confirmation of the presence or absence of a sexual stage in the life cycle of C. ribicola may have a future bearing on the economics of the chemical control phase of this project. Our lack of knowledge concerning the life cycle of this fungus is also an obstacle to current progress of our research. Certainly, present evidence concerning the means and source by which this fungus forms its true diploid stage is controversial (Colley, 1918; Hirt, 1964, and Pierson 1933). In addition, our inability to induce infected tissue cultures to produce aeciospores has hampered our ability to confirm macroscopically, that they are, in fact, infected with C. ribicola. An expensive and time consuming histological analysis is now required. Whether we have failed to satisfy the nutritional, environmental, or sexual requirements for aeciospore production is unknown.

Most of our proposed work in this area will be centered on the cultured, infected host tissue and the production of aeciospores therein. Additional work conducted on the cytology and ontogeny of the rust and that proposed under FS-INT-2302 should serve to adequately clarify this problem area for our purposes.

Of course, until such time that we can grow the rust fungus in pure culture, we have few alternatives for confirming the existence of strains of the fungus and studying their substrate specificity. Therefore, work centered on confirmed rust strains will be delayed. To date, FS-INT-2302 has identified two strains on the basis of needle lesion color, and others must exist.

Since we currently need methods to propagate the rust vegetatively in living hosts for fulfilling Koch's postulates with cultured isolates, as well as for providing uniformity of chemical testing materials and for examining the pathogenicity of strains whenever they can be established, we propose a specific study to develop this methodology. Preliminary tests have confirmed that the patch graft procedure (Patton, 1962) will work on western white pine although efficiency needs to be greatly improved. We have had limited success in inoculating living hosts with infected tissue cultures; and the infectivity of rust mycelium alone, taken from either infected tissue or pure cultures, is unknown.

Problem Area Outline

Current and proposed studies with their objectives are outlined as follows:

D. Life Cycle

1. Adaptive capacity to substrates

   a. 2303-___. Nutritional specificity of isolates. Objective: To ascertain the capacity of cultured isolates of *C. ribicola* to adapt to changes in their physical and chemical environment.

   b. 2303-___. Pathogenicity of isolates. Objective: To determine the differences in pathogenic characteristics of specific rust isolates.

   c. 2303-115. Vegetative propagation procedures for *C. ribicola in vivo*. Objective: To develop a technology for the artificial inoculation of the pine host (*in vivo*) with mycelium from *C. ribicola*.

2. Sporulation

   a. 2303-113. Factors affecting formation of *C. ribicola* aeciospores *in vitro*. Objective: To investigate and clarify the environmental, nutritional, and sexual factors affecting sporulation of *C. ribicola*.

   b. 2303-114. Stimulation of aecia production by *C. ribicola in vivo* in controlled environments. Objectives: 1) Define the duration and magnitude of chilling required for aecia production by rust infected western white pine seedlings and commensurate with acceptable growth by the seedlings, and 2) to determine if manual cross-fertilization of pycnia is beneficial to aecia production *in vivo* in controlled and modified environments.

   c. 2303-102. Chromatographic analysis of *C. ribicola* pycnial fluid. Objective: To separate and identify the organic compounds of pycnial fluid of *C. ribicola*.

d. 2302- . Sexual nature and genetic variation of C. ribicola (in vivo). Objectives: To develop an efficient method of obtaining infection from single basidiospore inoculations, controlled crossing procedures for the rust, methods for obtaining consistent and controlled aeciospore production, and more substantive evidence on whether C. ribicola is hetero- or homothallic.

3. 2303- . Cytology and ontogeny of C. ribicola. Objective: To study the life cycle of C. ribicola on P. monticola.

II. Integration of Biological Information

To embark on a properly based program geared to the search for rust controlling agents may be a long and expensive process. There is, however, at our disposal at the present time a wealth of knowledge concerning the process commonly referred to as "life" and its control, both within and without the natural confines of its environment. This information can and should be exploited in the area of plant disease control. It must be recognized that we are in fact dealing with the control of life and, in particular, the living processes that are responsible for maintaining conditions within the host-parasite system satisfactory for the survival and growth of the rust fungus (see Component 1-A and -B).

The possibilities are excellent that existing information will yield the key to control the vital functions of the host-parasite relations of rusts if we can understand these functions sufficiently well. The problem is to integrate this information and channel it toward our ongoing research.

This problem component has two primary facets: 1) Making current knowledge available in a form useful to the investigation, and 2) keying this information and integrating it toward specific ongoing project efforts. The testing and application of resultant information specifically obtained for rust control is proposed in Component III.

Development of an Information Retrieval System

A manual filing, retrieval, and integration system has been designed that temporarily satisfied many demands of the current problem as outlined. It utilized a specially designed edge-punched sorting card with 25 independent fields. However, we soon discovered that the complexity and scope of information required to satisfy project objectives rapidly exceed the capability of an edge-punch card system.

The following (partial list) of key words for information retrieval will give some idea of the complexity and scope of the information required that is available in the current literature.

analogue
precursor
mediator
stimulator
inhibitor
antagonistic
antimetabolites
antibiotics

induction
treatment
protectant
therapeutant
eradicant
protectant
chemotherapeutant
systemic

toxins
anti-toxins

enzymes
metabolites
adjuvant
resistance
acquired characters
immunity
premunity
additives
competitors
combator
transport
translocation
vitamin
growth factor
hormone
auxin
kinetin
rusticide
structure (chemical)
transport
sporulant
germinant
etc.

herbicide
pesticide

insecticide
fungicide
fungistat
mycostat
anti-fungal
anti-auxin
reaction kinetics
phytoalexin
phytotoxin
lactone
quinone
polyphenol
alkaloid
action (mode of)
movement
disease (control)
absorption
formulation
application
additive
anti-sporulant
anti-germinant
uncoupling agents

The physical plant capability and the expertise required to put together an automatic (machine) system based on current computer technology and capability are substantial. Therefore, we have negotiated an arrangement with the Computer Center and Department of Plant Pathology of Washington State University to fulfill this requirement. Though started in 1967, 2303-104, "An information service for chemical and/or biological control research and development studies on white pine blister rust," encompasses our most recent cooperative proposal with Washington State University, "An Information Service for Biochemistry and Control of Plant Parasite Systems, December 1968," to which the reader is referred for the technical details. The objective of this study is as follows: "To develop a retrieval system to provide relevant scientific and technical information on the specific fields of chemotherapy, host-parasite physiology, and biochemical differentiation in host-parasite complexes for the specific objective of exploiting the information bank to develop, sophisticate, and test, within the limitations of this information bank, hypotheses based on clues derived from ongoing research," thereby providing the project with possible control systems worthy of testing.

Integration of Retrieval Information

Research starts with a thorough literature search for information related to the problem. At present this requires a disproportionate amount of professional time. All too often with present methods, the search fails to reveal the pertinent information available. As a result, expensive research programs are repeated. In other instances research efforts are unproductive because effective techniques described in the literature are unknowingly overlooked.

Having an information bank and a means of retrieving that which is pertinent to start research is just a start. Of even greater significance is whether the retrieval system will effectively and efficiently direct ongoing research. As bits of data come from our research on _C. ribicola_, what does the current literature tell us about them with respect to continuing their study, stopping and directing our knowledge to application, or even backtracking an aspect of research. A few examples of specific information to be provided from queries of the system to facilitate ongoing research are:

1. Known enzymatic processes requiring a metal cofactor.

2. Control of such life processes as enzyme systems, respiration, nutrition, and related metabolic and anabolic cycles in fungi and plants.

3. Chemicals which control specific biochemical processes in fungi or in host-pathogen relations and which might be used as primary candidates in present tests to control blister rust.

4. Mode of action of these chemicals, factors influencing such action, and their translocation, persistence, and accumulation in plants.

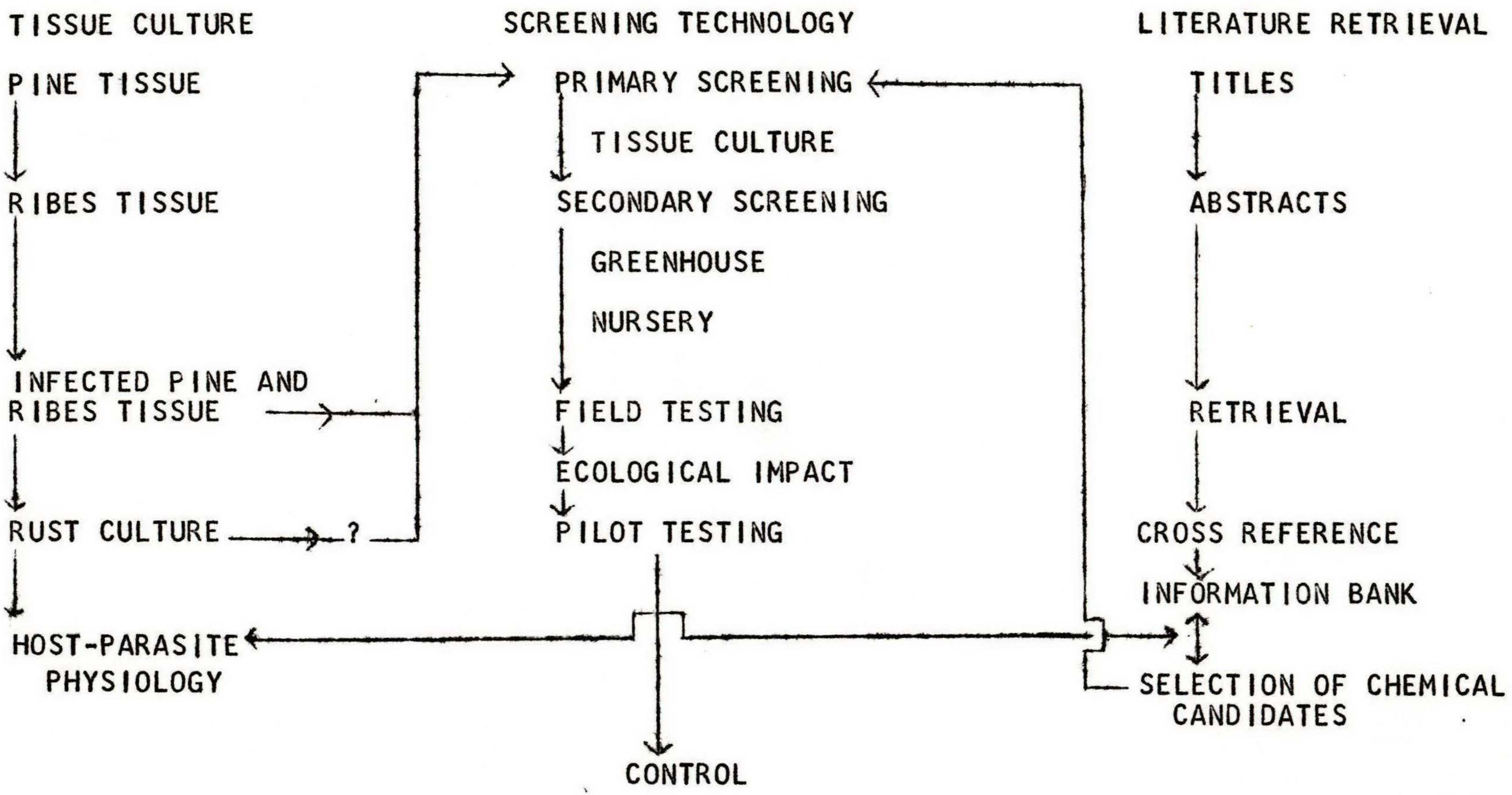
Taking example 3 above, we have proposed in Figure 1 how information obtained from our research on the biology of the rust (I-A) and from search of the information bank that identified a suitable chemical candidate is integrated in our Component on the application of information (III-A). Application of other biological information to other control measures would have a similar approach, though quite likely a different flow chart.

Success then is likely if the knowledge at hand and that yielded at the developing frontiers of the natural sciences, particularly in the area of host-parasite physiology, can be managed and properly integrated. The time and effort required to do this seems practical for the solution of the blister rust problem. However, regardless of the outcome concerning white pine blister rust, the accumulation and availability of the information will be well worth the effort and will be equally pertinent to other problems of the forest industries and to many other disciplines as well.

Problem Area Outline

Only one study is proposed in this Component and has already been identified with its objective (2303-104).

Figure 1. An Integrated Approach to Chemical Control of Blister Rust


TISSUE CULTURE
PINE TISSUE
RIBES TISSUE
INFECTED PINE AND RIBES TISSUE
RUST CULTURE
?
HOST-PARASITE PHYSIOLOGY
SCREENING TECHNOLOGY
PRIMARY SCREENING
TISSUE CULTURE
SECONDARY SCREENING
GREENHOUSE
NURSERY
FIELD TESTING
ECOLOGICAL IMPACT
PILOT TESTING
CONTROL
LITERATURE RETRIEVAL
TITLES
ABSTRACTS
RETRIEVAL
CROSS REFERENCE
INFORMATION BANK
SELECTION OF CHEMICAL CANDIDATES

III. Application of Biological Information in Control Measures

To this point we have proposed a program to provide a foundation of knowledge about the rust organism and its hosts and a means to mix that information in proper proportions for one specific purpose--to provide control methods for the rust disease. As we have said, eventual control may encompass several or all methods; and, although we discuss our program for testing biological, chemical, and silvicultural means, these can only supplement or be supplemented by genetic means.

Chemical

Most of the progress attained to date in chemical controls of plant disease has resulted from empirical searches for effective chemicals and the evaluation of these chemicals in the field. Tremendous success has been attained with protectant chemicals with more modest success in the search for eradicant chemicals. The search for translocatable eradicants, though enthusiastically pursued since biblical times, has as yet produced no commercially utilized agricultural chemicals.

Literature Review and Developmental Philosophy

With respect to the control of the blister rust organism, there appears to be three possible classes of chemical controls which might be economically feasible: 1) extremely long lived protectants for control on nursery stock, 2) locally effective (nonsystemic) eradicants for nursery stock and highly valued mature trees, and 3) a systemic eradicant for general usage, including possible aerial application on large areas of any age class. Control of the rust on the alternate host, or inhibition of sporulation may also be useful under some conditions if more effective means are not found. Obviously, investigation of the third class of chemicals would be the most useful and economical approach and will represent the bulk of the total investment.

Insofar as we know today, such a chemical could act in one or several of three ways: a) by increasing resistance of the host, b) by inactivating pathogen derived vivotoxins, or c) by acting as a systemic toxicant. No. c would be the most useful and economical for blister rust control. Therefore, although a and b cannot be ignored, it appears that top priority should be given to the search for a systemic eradicant which acts directly against the pathogen.

The search for such a chemical, because of its potential usefulness, has been going on since the days of the Roman Empire. Thus far, there are only two documented cases of the economical use of such a compound on a substantial scale, that of streptomycin for the control of fire blight (Zaumeyer, 1958) and 8-quinolinol sulfate for the control of geranium foot rot (Stoddard, 1957). Since 1949 the search for systemic eradicants of plant diseases has been intensive (Stoddard, 1951, 1957; Weindling, et al., 1950; Horsfall and Dimond, 1951; Horsfall, 1956; Dimond and Davis, 1952; Dimond, 1958, 1965; Dimond and Horsfall, 1959; Crowdy and Pramer, 1955; Crowdy, 1959; Zaumeyer, 1958; Livingston and Hilborn, 1959; Oort and Dekker, 1960; Oort and van Andel; 1960; Sijpesteijn, 1961). Less extensive efforts specifically geared for the control of plant rusts have also been made (Dickson, 1959; Rowell, 1968). In addition, considerable effort has been made to chemically alter many compounds which have shown promise in the field of chemotherapy (Byrde, et al., 1953; Crowdy and Wein, 1951; Fawcett et al., 1955; Van der Kerk, 1956). Although improvements have been made, they have obviously not been adequate to provide the success required. Some antibiotics have reduced disease severity (Brian, 1954; Zaumeyer, 1956) but have not been widely used.

Why then, with all this work, do we have so little success, particularly in view of the fact that a great many systemic insecticides have been used commercially with success for over 10 years? The answer to this question is probably simple. In controlling insects on plants one is working with widely divergent living systems. The insect, being an animal, is more highly evolved, better understood, and generally has a far different and more narrow ecologic amplitude than the plant. Therefore, an empirical search for systemic toxicants has a reasonable likelihood for success. On the other hand, control of a fungus disease on a plant requires differentiating between two relatively similar systems, i.e., a plant on a plant. In addition, fungi as a rule contain a broad spectrum of genetic types and are highly adaptive. Consider also that the host is the more complex and highly evolved component of the host-parasite system and may, therefore, be the most susceptible of the two to broad spectrum toxicants. Thus, the likelihood of discovering chemicals toxic to one plant system but not the other via an empirical approach is very small. Under these circumstances, how then does one approach the search for chemicals selectively toxic to the blister rust fungus?

Since the blister rust fungus is a member of a class of organisms considered obligate parasites, it therefore may be adapted to survive only under conditions provided in the living host. Thus, it belongs to a unique class of fungi whose requirements are highly exacting. The fact that the blister rust organism and the pine are not similar either morphologically or physiologically is reason enough to surmise that their vital processes are dissimilar and that knowledge concerning the host-parasite system will render the rust controllable. Thus, if we can uncover exactly what the host is supplying to the rust fungus and enabling it to survive in the host-parasite complex and if we can understand how this is being accomplished (Component I-A and -B), we have only to develop a means of identifying those chemical candidates potentially capable of interrupting this system (Component II) and means of testing their effectiveness in this Component in order to control this rust (Figure 1).

One other aspect is particularly significant in applying the information sought on the specificity of the host-parasite system (I-B) to our selection of chemicals. The side effects of chemicals are of considerable importance to anyone proposing their application to forest stands. Therefore, if we select chemicals which function specifically on a particular system in the host-parasite metabolism, we automatically impose a defined set of parameters referent to possible side effects on other organisms; and the scope of any future work in this field will be defined. Conversely, use of broad spectrum chemicals would impose a considerable work load merely to explore the potential range of possible side effects.

Current and Proposed Research

As documented in our previous problem analysis, evaluation of rust control in the field is seriously hampered by our inability to determine the true viability status of rust cankers, the extreme variability of the natural environment, and our dependence on time as an indispensable factor in evaluation. Preliminary examinations of infected nursery stock and greenhouse grown seedlings indicate that these same problems will be applicable here also and the only meaningful data will probably be based on tree mortality. Therefore, our primary testing phase must be highly reliable and capable of determining the true effect of any given candidate on the rust fungus. We must have a good possibility of establishing control of the rust with any candidate which is to be entered into a testing program based on the highly variable natural infections of host trees of any age. Otherwise, cost of evaluating chemicals will rapidly exceed the economic capability of the project and will cause other aspects of our effort to suffer for a program which can test only a small number of materials having only an infinitesimal chance for success.

Therefore, the bulk of our immediate effort directed to testing technology (2303-95) will be concentrated in the primary testing phase and will be extended into other phases only as required until we are firmly convinced our primary phase is reliable. From the outset of the program, it was hoped that we could culture the rust fungus, under defined conditions, via a system that closely resembled natural conditions. Culture of the fungus on artificially propagated host tissues (Harvey and Grasham, 1969, 2303-93) now provides us with this technology. The high success ratio in propagating infected host cambium (90% of all explants produce callus and 70% of these produce extensive growth of the rust fungus) provides a reliable system of propagating the parasite under conditions where it remains dependent, as it is under natural conditions, on host tissue. Thus, we can test candidates, on the host-parasite system, under highly defined conditions, subject only to those variables we desire to retain.

Obviously, the physical and chemical environments required for the culture of this host-parasite complex have to be defined and be retained in a constant state. In addition, single mature trees can provide hundreds of cultures which can be subdivided, when cultured, into thousands--so host material can, if desired, remain rather constant. By transmitting clonal lines of the rust, derived from a single canker or spore, it also can remain constant. Thus, we can maintain the entire system under rather closely defined conditions.

Alternatively, since a wide variety of host and fungus genotypes exist in nature and control will ultimately be performed on them, we may require a broad, highly variable base, i.e., infected callus isolates obtained from a large number of cankers. Firm decisions on the degree of rust and host variability required in the primary testing phase must await additional experience in the actual testing of chemicals and assessment of the variability of data obtained. But at this time, it appears reasonable to assume that the reliability required of this phase will require maintaining as broad a biological base (host and parasite) as is commensurate with reliable test data.

When we have established a base for primary testing, chemical candidates will be our primary concern. As indicated, these would ultimately be chosen through searches provided by the proposed information retrieval service. Many factors must be considered with respect to relationships to host, parasite, and the host-parasite interaction in the search for potential candidates for use in blister rust control. In order to be tested in our system we propose that any candidate must have one or more of the following two characteristics recorded in the literature:

1. Known or suspected metabolic function related to the host, the parasite, or their relationship to each other.

2. Known or suspected high potency fungicide, bacteriacide, phytocide, etc., with chemical characteristics suggesting a possibility of at least localized systemic action.

As an alternative to an eventual retrieval system, 135 known pesticide manufacturing firms in the free world have been canvassed during the past year to determine what materials would be available to begin developing our primary testing phase. Of the 40 chemicals supplied by these firms, five classes of compounds will pass the two criteria described above and will be used to gain experience in primary testing. The six most promising of these chemicals are now being used to process the primary testing phase. For academic interest and comparative purposes, Phytoactin and cycloheximide may eventually be tested with this system.

When and if we find chemicals which can reliably and completely eliminate the rust component from cultured infected tissues in the primary test phase, they will be carried through successive testing phases which will include greenhouse and/or nursery phases on tree seedlings and field evaluation on trees before pilot testing is attempted. Although all testing phases are provided by 2303-95, supplements will be provided for each step. With this system we should have a high likelihood of success at any testing level before such testing is attempted, thus, hopefully eliminating investment in low return screening projects.

Problem Area Outline

Since pilot testing would likely be tests of one chemical either in or not in conjunction with other treatments in cooperation with State and Private, R-1, no specific studies are identified. The other two are briefed with their objectives as follows:

A. Chemical

1. 2303-____. Selection of chemical candidates. Objective: Using the information retrieval service to provide lists of chemicals having specified attributes to be used as test candidates in the primary test phase.

2. 2303-95. Development and implementation of an assessment program for screening potential therapeutic agents against white pine blister rust. Objective: To assess the potential of

promising therapeutic agents for white pine blister rust control.

3. Pilot testing of promising compounds.

Biological

Biological control is very appealing from the standpoint of procedures, costs, and the absence of residue problems. However, experiences with biological control of plant diseases have been anything but encouraging. Not a single success can be counted. On the contrary, the field of entomology has enjoyed some spectacular successes with the biological control method, particularly against introduced insect pests. Yet, biological agents are known to affect fruiting, growth, and development of several plant pathogens, some of which are obligate parasites. Few, if any, of these biologic agents have been studied in the detail required to properly evaluate their potential as control agents because plant pathologists have not been attracted by the biological method of control, control is relative, and biological control is difficult to appraise. Most of the knowledge acquired is the result of effort expended because of mycological curiosity and motivation. Thus, the majority of information which we have available concerns structure of the active agents and not function and impact which is the information we need, as plant pathologists, to evaluate their potential as control agents.

In this analysis we not only propose evaluation of specific biological agents but appraisal of the impact of total inactivation. The contribution of each factor to the total cannot be identified in the field; but, by studying each factor, we may be able to determine individual or specific group contribution to the total. Since literature on both phases of this problem area occasionally overlap it is reported subsequently with primary emphasis on _Tuberculina maxima_.

Suppression of native rust fungi throughout the world is due, in part, to their natural enemies which are primarily other fungi. Arthur (1929) lists 37 fungi attacking rusts or living in intimate association _in vivo_ with them. One fungus, _Darluca filum_ (Biv.) Cost., is associated with at least 71 species of rust in Latvia alone (Kirulis, 1942). However, of the many fungi known to associate with the rusts, _Tuberculina_ spp. are the most effective at inhibiting their normal growth and development (Tubeuf, 1901, 1930, 1936; Lind, 1913; Weir and Hubert, 1917; Wilson, 1925; Brooks, 1928; Deacon, 1939; Hulea, 1939; Thirumalachar, 1941; Kirulis, 1942; Rayss, 1943; Keener, 1954; and Barkai-Golan, 1959).

Among the first fungi reported associated with white pine blister rust cankers was the purple mold, _T. maxima_. This Deuteromycete was described by Rostrup (1890). Thbeuf (1901) discussed the taxonomy and host range of the genus _Tuberculina_ and reported _T. maxima_ capable of attacking the aecia of *Peridermium strobi* (*Cronartium ribicola*). In 1913, Tubeuf (1914a, 1914b) asked the question "Is *T. maxima* able to destroy (control) blister rust of white pine?" His observations reported in 1914 and those of his student Lechmere (1914) did not fulfill their expectations. Lechmere (1914) clarified the life history of *T. maxima* and considered it a typical example of double parasitism. He concluded that only the pycnia and aecia could be attacked; that the parasite cannot invade the pine tissue itself and destroy the rust mycelium and therefore could not be employed as a biological control. These conclusions were based on only a few observations; at least, no experimental data were given. Tubeuf (1930) and Rohmeder (1931) concluded that one does good by using *T. maxima* to combat the blister rust disease.

The purple mold was first reported in United States by Weir and Hubert (1917) on native pine rusts in Montana and was first found on blister rust in North America on western white pine at Daisy Lake on the coast of British Columbia in 1926 (Mielke, 1933). Until 1932 it had been found on this host only along the coast of British Columbia where it also occurred on native *Cronartium comptoniae* on lodgepole pine. In 1932 it was found on white pine blister rust for the first time in United States at Newman Lake, near Spokane, Washington (Mielke, 1933; Hubert, 1935), where the blister rust was first found on pine in the Inland Empire in 1928 (the rust became established here and at other locations in the Inland Empire in 1923). In 1932 the purple mold was also found in the interior of British Columbia on western white pine rust cankers (Mielke, 1933).

Although early observations on the purple mold's ability to control the rust on western white pine were not encouraging (Goodding, 1932; Hubert, 1932; Mielke, 1933), each of these early workers expressed the possibility that the mold could develop a strain that would be more virulent on the rust. Hubert even obtained from Germany in 1931 a strain of *T. maxima* that he hoped would be able to control the rust here as it had in some parts of Germany. He inoculated rust cankers at two localities in Idaho, but by 1934 this mold strain from Germany, as well as material obtained in British Columbia in 1931 and 1932, had given unsatisfactory control results (Hubert, 1935).

The early work with *T. maxima* on the rust of western white pine as well as the European work on other pines demonstrated the ability of the purple mold fungus to parasitize rust cankers. In the Pacific Northwest, Goodding (1932) reported he found the purple mold "often completely destroying aecial production." Hubert (1932) stated, "Every canker showing purple mold gave evidence that aecial sporulation had been arrested." His concern was not with the effect of the parasite on the rust cankers, but with its apparent inability to spread to enough cankers to appreciably reduce rust damage. Mielke (1933) stated, "The parasite attacks both pycnia and aecia of the white pine blister rust. In British Columbia it has been observed that production of the aeciospores is greatly reduced when the aecia are attacked and that death of the bark over the cankered area often occurs in a few years." In Germany, Tubeuf (1930) found by inoculation that _T. maxima_ suppressed formation of aecia and repeatedly led to the healing of the aecial stroma in pine.

Mielke (1933) observed that in British Columbia pycnia may be attacked throughout the entire seasonal period of their development, production is reduced, and a pycnial zone attacked one season usually fails to produce aecia the following year. Spaulding (1929) observed in Europe, where _T. maxima_ occurs, that pycniospores are not produced in great abundance. A study made at Oregon State University showed that an aqueous solution of the pycnial fluid is an excellent medium for the germination of _T. maxima_ spores (Mielke, 1933). Because of the preference of purple mold on pycnia, he suspected that its effect on aecial production was indirect. He substantiated this by shaving off the outer bark of the parasitized portion of a stem canker and one month later observed _T. maxima_ sporulating profusely on the cut surface. He interpreted this to demonstrate that the purple molds' mycelium invades the bark well beneath the pycnia and aecia on the surface. He believed that this may explain why this fungus appears to hasten the death of infected bark.

The possibility that biological components of the microflora of blister rust cankers, other than _T. maxima_, may have a pathological effect on the canker has not been overlooked. However, investigation of such has attracted even less attention than has _T. maxima_. While several fungi are reported to inhabit blister rust cankers (Tubeuf, 1901; Posey and Gravatt, 1917; Colley, 1918; Rhoads, 1920; Snell, 1929a, 1929b; Goodding, 1932; Wollenweber, 1934; Hubert, 1935; Bingham and Ehrlich, 1943) information on the nature of their association with such cankers is essentially nil. There is a very notable void of reports of fungal inhabitants of blister rust cankers, other than _T. maxima_, from Europe and Asia.

In a study of the fungi associated with blister rust cankers of western white pine, made in 1941 in the Inland Empire, Bingham and Ehrlich (1943) reported twenty-four different perfect and imperfect stages of fungi. Only one of these,

T. maxima, was considered to be parasitic on the rust fungus itself. The others were considered weak bark parasites or saprophytes which subsist on the weakened or dead bark of the blister rust cankers. These bark parasites starve out the rust by killing the cankered bark, upon which the rust fungus is dependent for its sustenance, while T. maxima parasitizes the pycnia and aecia of the rust fungus itself (Bingham and Ehrlich, 1943).

On each of 13 sample strips examined during their study Bingham and Ehrlich (1943) estimated 0-14 percent of the rust-threatened trees had been saved through intercession of the canker fungi. They believed the smallness of these percentages was due, in part, to the intense pine infection and the small size of the reproduction on the selected strips, where the proportion of stem cankers was particularly high. Each such stem-cankered tree constituted a threatened tree, the deliverance of which could not be effected. This fact was well demonstrated in two strips where more than 70 percent of the potentially damaging branch cankers were parasitized and judged dying but where only 0 or 2 percent of the threatened trees could be considered saved by the parasitic canker fungi. Where trees are larger or the rust infection not so intense, they concluded the less numerous direct stem infections should increase the effectiveness of the canker fungi in saving rust-threatened trees. Their data showed that the percentage of canker parasitism is dependent more on age and stage of the cankers than on intensity of pine infection.

On their 13 sample strips, Bingham and Ehrlich (1943) found aecial sporulation reduced 5 to 60 percent by canker parasitism. They found a high and significant degree of direct correlation of aecia reduction with the percentage of cankers parasitized. Volume of aeciospores is reduced more by parasitism than is percentage of aecia; as one-year sporulating cankers, which produce relatively small volumes of spores, were parasitized 32.5 percent while 3-year sporulating cankers, which produce relatively large volumes of spores, were parasitized 57 percent.

Bingham and Ehrlich were much more enthusiastic about biological control of blister rust in western white pine from their 1941 studies than earlier workers had been 9 or 10 years before. Although they did not determine the intensity of canker parasitism by T. maxima separately in their studies, evidently Bingham and Ehrlich (1943) found it occurring commonly, compared to its extreme rarity 10 years previously. However, they did not find T. maxima to be in the four most common canker parasites encountered in the studies. Now, 23 years after Bingham and Ehrlich's observations, T. maxima is undoubtedly by far the most commonly encountered canker parasite on western white pine blister rust cankers in the Inland Empire region. No one, apparently, has made a study of *T. maxima* on western white pine cankers since Hubert's work 34 years ago, or no one has made detailed tabulations of its occurrence and blister rust control effects in the Inland Empire since Bingham and Ehrlich's study 26 years ago.

Evaluation of Biological Agents

In recent years, field observations and data reported by several workers indicate an increasing degree of biological inhibition of blister rust cankers on western white pine growing in the Inland Empire. The accumulated evidence that the microflora (primarily fungi) which inhabit blister rust cankers may have an adverse effect upon growth and development of such cankers, either directly or indirectly, supports this as factual. Although incomplete, our most recent data lists at least 40 presumably different fungi inhabiting western white pine blister rust cankers in the Inland Empire, with both perfect and imperfect stages of some being commonly encountered. Some of these are mere epiphytes, some are saprophytes, some are parasites and a few are pathogens. It is the latter group with which we are concerned.

The published literature, unpublished observations by professional personnel, and our field and laboratory data from work initiated since 1965, indicate that the lilac fungus, *T. maxima*, is the most active biological agent having a *definite* and demonstrable pathologic effect on blister rust cankers. This imperfect fungus reduces the inoculum potential of *C. ribicola* by suppression of both pycnia and aecia production *and inhibits* growth and development of the cankers, which results in partial or, more rarely, complete inactivation of such cankers (2303-75 and 77).

The primary question, "Is *T. maxima* effective and, thus, of some value as a control of *blister* rust?", is of interest to all concerned with management of white pines susceptible to the blister rust disease. Secondary questions are, "What is the effectiveness of other canker inhabiting fungi?", and "Can any of these fungi, including *T. maxima*, be used in combinations to produce *significantly higher* levels of control than the individual can?" Thus, our analysis emphasizes a thorough evaluation of other canker inhabiting organisms as potential biological control agents, once their identity is known.

Current Work

Operating under the 1965 problem analysis, we have initiated 10 studies for purpose of acquiring basic information on the biology of _T. maxima_ and its association with western white pine blister rust cankers. Four of these studies have been completed and the results published or are in the process of being published. One study (2303-74) has been reorganized and incorporated in a new study (2303-91). Some preliminary results available from our other studies will be related in the persuing discussion. Additional information on _T. maxima_ has been acquired from a few exploratory experiments which have not been formulated to the point of becoming official studies and perhaps do not warrant such status. The interest which our planning, current research effort, and personal contacts have stimulated in our contemporaries within and outside the Forest Service in the western United States and Canada has proven rewarding in terms of knowledge gained concerning _T. maxima_ and its effect on blister rust cankers.

From the knowledge provided by the literature on biological control of plant diseases and from knowledge and experience gained from working with biological control agents of the dwarf mistletoes (_Arceuthobium_ spp.), six attributes of a biological control agent are considered essential (Wicker and Shaw, 1968). These attributes are (a) distribution which coincides with that of the target pathogen, (b) ecologic amplitude sufficient to ensure persistence within its host range, (c) production of abundant inoculum (spores) for establishment of epiphytotics, (d) high infectivity, (e) high virulence, and (f) an efficient mode of action for curtailing development of target disease. The success of such a control agent is predicated upon its possession or acquisition of these attributes. Therefore, these attributes provide a basis for evaluating the potential effectiveness of biological control agents. Thus, our proposal for evaluating the effects of _T. maxima_, and subsequently other agents, on blister rust cankers and its potential use in a control program is formulated around these attributes.

Results from our survey (2303-74) in 1965 provide information on the distribution of _T. maxima_ within the confines of the Inland Empire (Kimmey, 1969). _T. maxima_ was present on western white pine blister rust cankers at all 48 plot locations on the Clearwater, Coeur d'Alene, Colville, Kaniksu, and St. Joe National Forests. The fungus was actively fruiting on 24.3 percent of the active lethal-type cankers. _T. maxima_ fruits only on active cankers; it is not possible at present to ascertain accurately its past occurrence on cankers. Aecial production was reduced to 14.2 percent for the active lethal-type cankers and to 5.4 percent for the entire lethal-type canker population. We do not claim this to be entirely the result of the action of *T. maxima*, but a large percentage of it is undoubtedly associated with *T. maxima* infection, because the fungus was fruiting on 1.4 percent of the cankers producing normal aecia, 39.7 percent of the cankers producing partial aecia and 25.2 percent of active cankers producing no aecia. Our inability to recognize the presence of *T. maxima* in the absence of fruiting certainly masked the relation between this fungus and aecial production.

Recent surveys, mycological collections, and personal observations indicate that *T. maxima* is also well established in California, Oregon, Washington, Idaho, Montana, Wyoming, British Columbia, Alberta, and Northwest Territories on rusts such as *Cronarbium ribicola*, *C. comandrae*, *C. comptoniae*, *Peridermium harknessii*, and *P. stalactiforme*. There are undoubtedly other Uredinaceous hosts of the fungus within the area mentioned. As interest increases, these associations will be observed and recorded; and, certainly, the geographic distribution of the fungus in western North America will be found much greater than current reports indicate.

T. maxima has not been reported in association with uredial or telial sori of *C. ribicola* on *Ribes* spp. Tubeuf (1901) reported collections by several European workers (Gobi, Frank, Magnus, Schröter, and Rostrup) of *Tuberculina* spp. occurring on uredia and telia as well as spermogenia and aecia of several rusts. However, in all cases where enough information is provided to permit a check on the nature of the life cycle of the rusts, the collections of *Tuberculina* spp. reported in association with uredia and telia were on autoecious rusts, regardless of whether they were macrocyclic, demicyclic, or microcyclic. In these cases, infection by *Tuberculina* spp. very likely occurred during pycnia and/or aecia production and remained throughout uredia and telia production. One collection listed by Tubeuf (1901) reports a species of *Tuberculina* on *Darluca filum* (Biv.) Cast., another imperfect fungus associated exclusively with the Uredinales. Gobi (1885) reported *Tuberculina* sp. on *Paris quadrifolia* without finding a Uredinales on the host plant. Undoubtedly a rust was present, Gobi just failed to detect it.

We have successfully isolated *T. maxima* from the discolored margin of "dormant" cankers collected from the field during the winter months (December-March). We now know that *T. maxima* can overwinter on western white pine blister rust cankers as spores, sporodochia, and as mycelium within the pine cortex (Wicker and Wells, 1968). Continuous freezing for periods up to 19 months does not completely destroy spore viability. Thus, T. maxima is well adapted to overwintering in the imperfect stage and viable inoculum is available, under natural conditions, for invasion of blister rust cankers any time the cankers are susceptible and the environment is favorable. Also, reinfection is not necessary on an annual basis. These data and our many field observations indicate that the mycelium of T. maxima does ramify throughout the cankered pine tissue and is not restricted to the aecia and pycnia as Tubeuf (1914a, 1914b) and Lechmere (1914) reported. T. maxima does not go beyond the rust invaded pine tissue and, thus, does not constitute a canker and subsequent threat to the tree in itself.

T. maxima can only infect western white pine blister rust cankers during pycnia and aecia production. Non-fruiting cankers (juvenile or fruiting retarded) could not be infected even following scarification of the discolored canker tissue with carborundum (Wicker and Kimmey, 1967). Data from our artificial inoculations of blister rust cankers with spore suspensions of T. maxima both in the field and under controlled conditions in growth chamber (2303-78) show the incubation period to be 2-7 weeks for both aecia and pycnia inoculations. The incubation period is defined as the elapse time between inoculation and subsequent spore production. Thus, the incubation period encompasses both the penetration and infection periods. It is quite evident that T. maxima is capable of completing its life cycle in a very short period.

We can grow T. maxima on a variety of artificial media. We are currently investigating the nutritional requirements of the fungus (2303-83). The preliminary data indicate that the fungus can utilize numerous carbon and nitrogen sources. However, there are some it cannot use and still others that may even be toxic, particularly, nitrite compounds. We know that T. maxima grows exceedingly well in pycnia fluid and that pycnia fluid of C. ribicola has a high fructose content and contains several ninhydrin positive nitrogenous compounds (2303-102). We also know that growth of the fungus is favored by a high carbon content with the optimum C/N ratio being 20:1.

Our investigations of the ecology of T. maxima (2303-84) have defined the temperature range. Spores germinate at 5° C with little or no additional growth. The thermal death point is between 25-30°C as no growth occurs at 30°C. The optimum temperature appears to be between 20-25°C and varies slightly according to the medium used. Our studies of pH are in process and so far we have grown the fungus on media from pH 3.0-8.0. The optimum is around pH 5.0.

During our recent (1965-1967) field evaluation of cycloheximide and phytoactin as chemical controls for the blister rust disease (2303-50), initial observations indicated that these two antibiotics were suppressing *T. maxima* at the concentrations used when applied directly to the canker surfaces. Our data from both field (2303-50) and laboratory (2303-85) studies confirm both antibiotics to be phytotoxic to *T. maxima*. When tested *in vitro*, cycloheximide completely inhibited *T. maxima* at 1-1000 ppm. Complete inhibition occurred with phytoactin at concentrations >70 ppm (Wicker, 1968). Thus, our data suggest that direct canker treatment using these two antibiotics may have been detrimental to blister rust control rather than just ineffective (Leaphart and Wicker, 1968; Wicker, 1968).

Proposed Research

We have made some progress during the past three years in our effort to gain a better understanding of the biology of *T. maxima* and to evaluate its potential as a control for the blister rust disease, but much remains to be learned before we can render a verdict on the exploitation of its control potential. We propose to continue our investigations formulated to provide the information required to evaluate *T. maxima* in accordance with the six attributes of a successful biological control agent which were discussed earlier. Once we have made this evaluation, we are then in a position to recommend possible modifications in the pine-rust-*T. maxima* association which might enhance the ability of *T. maxima* as a potential control measure. Therefore, we propose the following research effort on *T. maxima*, shown in descending priority:

A. Complete the existing studies.

B. Investigate the mode of action of *T. maxima* on blister rust cankers at tissue and cellular levels.

C. Investigate pathogenicity and parasitism by *T. maxima* in this triple association.

We are currently beginning to investigate the mode of action of *T. maxima* on blister rust cankers. There is no evidence of direct parasitism of rust spores or mycelium and, thus, it is incorrect to refer to *T. maxima* as a hyperparasite of *C. ribicola*. Preliminary data from histological investigations of *T. maxima* invasion of rust-infected pine cortex and rust-free pine callus indicates that the action of *T. maxima* in both tissues is the same; the pine cells are digested (Wicker and Woo, 1969). We suspect the rust mycelium, when present, is likewise digested. The evidence suggests that *C. ribicola* predisposes the pine cortex to invasion and digestion by *T. maxima*. Our evidence implicates the middle lamella as being the critical area.

We need to know why _T. maxima_ can effect this pathological condition of the rust-pine association. To know this, we must know how and why the rust affects the physiology and morphology of the pine tissue. There is definitely a succession of biological events occurring at tissue and cellular levels in this triple association which we need to elucidate in order to answer the how's and why's of the association and to evaluate the potential effectiveness of _T. maxima_ as a control measure.

Finally, we need some idea of the range and magnitude of the natural variation and the adaptable capacity for pathogenicity and parasitism that exist between and within different isolates of _T. maxima_ and different white pines. This information is directly related to the degree and rate of the pathological effect which _T. maxima_ has on the rust-pine association and these are key factors in the effectiveness of _T. maxima_ as a control for the blister rust disease.

Proposed research on other biological agents is broad in scope and preliminary to detailed studies. As we find insects on cankers, we propose only to identify them and describe their association (and role if possible) with cankers. Principal effort will be directed to other fungi with the intent to study them in detail as for _T. maxima_, provided they demonstrate potential for control.

Investigations on other fungi should be segmented for _C. ribicola_ geographically; namely, (1) in the western white pine type, (2) in the western U.S., (3) in the entire U.S., (4) in foreign countries. We have already started investigations of the microflora of blister rust cankers within the western white pine type under a cooperative agreement with Washington State University (2303-94). Under this agreement, fungi and bacteria are being isolated from cankers, grown in pure culture, and identified. Presently, over 40 fungi have been isolated. These are now being used to inoculate the rust-pine association in both the field and laboratory for purpose of obtaining information on the relationship of these fungi to rust cankers. These investigations are in their third year of progress and should be continued for at least one more year so that we can obtain some basis for screening for potential control agents.

It is also proposed that similar investigations be initiated within the other three geographic areas outlined. Since the rust fungus is exotic to North America and of recent introduction in terms of natural selection, mutations, and evolution, search for a biological control agent would likely be most fruitful in the country of the rust's origin or countries where it has been present for several centuries. Therefore, geographic area (4) should be the first place to start investigations and we will start these in 1970. This effort might be subsequently supplemented by study through IUFRO/FAO, under PL #480, or possibly under the Mutual Security Act 68 Statute 832. Investigations in geographic areas (2) and (3) could be accomplished through cooperative research programs with Universities and Colleges within the areas, as we have done with (1).

Evaluation of Natural Inactivation

Our observations in 1965 in young stands not treated with antibiotics indicated a high level of natural inactivation. Many factors may singly or collectively cause this inactivation. Irrespective of cause, both the permanency and change in status of canker inactivation, i.e., its status and significance over time, must be assessed. Variations in canker habits between sites indicate the need to assess the ecological factors affecting natural inactivation. Considered collectively under "impact," this aspect of biological control is separated in our analysis from study of individual agents such as *T. maxima*. Natural inactivation, for our purposes, may be considered as the summation of control contributed by all natural agents.

As has been previously pointed out in this problem analysis, the only method we have of assessing canker viability is macroscopic observation of canker activity over time. This fact points to a need to develop a technique to speed up the evaluation of control procedures. On a broader scale, we also recognize forest management need for more reliable and rapid disease detection methods for scaling individuals and/or stands.

Recent advances in infrared technology coupled with better knowledge of host-parasite relationships indicate good possibilities in applying remote sensing techniques to our problem of viability determinations of individual cankers. Previous studies by our project using histological techniques were unsuccessful in assessing *C. ribicola* activity. Laboratory procedures such as cultural techniques are not readily adaptable to field use while remote sensing techniques would be very suitable.

Current and Proposed Research

From the 1965 survey (2303-74) of 40 lethal type cankers at each of 48 locations in stands in northern Idaho, western Montana, and northeastern Washington not treated with antibiotics, we found that 62 percent of the cankers were inactive when examined (Kimmey, 1969). Should these cankers have remained inactive and healed over, one-third of the trees, then lethally infected, would have been saved from death by blister rust through intervention of natural control. Significantly, 38 percent of all bole cankers were also considered to be inactive.

In 1966, 2303-74 was revised to put our data on a crop tree basis and to establish annual levels and trends of inactivation (2303-91) and permanency (2303-92) on a minimum of 10 lethal cankers on a minimum of three trees on each of 49 plots. All non-lethal cankers are evaluated also on these special study trees. The influence of natural inactivation on rate of tree mortality will be studied on 20 trees selected on most of these plots. In contrast to the 1965 data, 16 percent of the lethal cankers in this new population were inactive in 1966 and 25 percent in 1969. We also have found that cankers frequently change activity status. Much variation in levels of inactivation between plots has been evident, suggesting that environmental factors may influence inactivation. Canker age has been shown to be correlated with canker inactivation, i.e., the older the canker the more likely it is to be inactive (Kimmey, 1969).

The present level of natural control could increase, decrease, or remain relatively static. In any case, we do not know what the destiny will be of a canker judged inactive today. Such knowledge is necessary (1) to properly assess the effectiveness of control measures we might initiate, such as chemicals, (2) to provide a basis for measuring the significance of biological agents acting individually or in interaction, and (3) to chart future management actions, e.g., the need for supplemental controls. Although cankers used in our two current studies on inactivation have been examined biannually through 1968, they have subsequently been scheduled for annual examinations. Some plots will receive rather intensive study to catalogue canker development at short intervals throughout a year.

Our knowledge of the microflora and -fauna of blister rust cankers, host physiology, resistance, and other environmental factors, as related to canker inactivity, is extremely meager. Because inactivation varies by plot and trees within plots, we propose study of site factors in relation to this phenomenon as the initial step to assessing its cause (see I-C-2-b). Necessity for further study will depend on the significance of natural inactivation over time as well as the clues from site relationships.

In a progress report by the remote sensing project (FS-PSW-2204), Wear (1966) reports use of a radiometer in detecting long-wave infrared radiation from Poria weirii infected trees, not yet showing crown symptoms. These trees exhibited 2 to 4°C higher temperatures than neighboring non-infected trees.

In our study of blister rust, infrared ektachrome has been used to photograph certain cankers at the time visual examinations were made in 2303-91. Normal ektachrome photographs were taken concurrently. The conclusion is that infrared film does not show anything about canker viability that cannot be seen on normal ektachrome. However, in many cases the contrast between certain portions of the canker will be enhanced by the infrared film.

Hackforth (1960) and Conn and Avery (1960) cover the basic principles of infrared radiation and its application. This indicates possibilities of determining canker viability because of differences in absorption characteristics of different bodies. Hackforth discusses a variety of detectors and optical materials available. Rapid advances in infrared technology have made available new devices and components that are more sensitive.

Our study of energy relations (2303-119) will provide the basis for the three remote sensing studies proposed here. However, rather than undertake them ourselves, we would hope that the remote sensing project (FS-PSW-2204) with their technical capabilities would take over the equipment design and evaluation studies with our cooperation.

Problem Area Outline

The following outline lists current and planned studies with their objectives in the two major subdivisions of this problem area. While the research on natural inactivation is new, that on the biological agents is largely an updating of our original analysis. As for other problem areas, pripirity of study in both is shown in Table 1 in the same format listed below.

B. Biological

1. Evaluation of Biological Agents

a. Tuberculina maxima

(1) Biology

(a) 2303-83. Investigation of the nutritional requirements of *T. maxima* Rostrup *in vitro*. Objective: To investigate nutritional requirements of *T. maxima* and to record its cultural characteristics.

(b) 2303-84. Ecology of *T. maxima*. Objective: To investigate the ecology of *T. maxima* growing *in vitro*. (The effects of environmental factors such as light, temperature, and pH on growth, development, sporulation, viability, and pathogenicity of the fungus will be evaluated; and minimum, optimum, and maximum parameters will be established where practical.)

(c) 2303-78. A plan to determine the incubation period of *T. maxima*. Supplement #4 to 2303-74. Objective: to determine how soon, under measured temperature and humidity conditions, after a susceptible blister rust canker is successfully inoculated with viable *T. maxima* spores that the infection becomes macroscopically visible, i.e., produces new *T. maxima* spores.

(e) 2303-____. Host range of *T. maxima*. Objective: To determine the host range for *T. maxima*.

(e) 2303-____. Spread of *T. maxima* in nature. Objective: To investigate spore maturation, dissemination, and viability under natural conditions and identify the agent(s) responsible for dissemmination.

(2) Effectiveness

(a) 2303-75. The effects of *T. maxima* on white pine blister rust cankers. Supplement #1 to 2303-74. Objective: To collect factual data on the development and effects of naturally occurring *T. maxima* on blister rust cankers of western white pine, and to compare this with its development and effects on similar cankers treated with antibiotic materials.

(b) 2303-77. Time required for *T. maxima* to inactivate blister rust infections on western white pine. Supplement #3 to 2303-74. Objective: To determine the time required, after its establishment, for *T. maxima* to inactivate sporulation and growth of blister rust cankers of various sizes and ages on western white pine.

(c) 2303-112. Mode of action of *T. maxima* in the inactivation of white pine blister rust cankers. Objective: To determine how *T. maxima* functions within the rust-infected pine cortex at the tissue and cellular levels with respect to both the pine and the rust.

(d) 2303-____. Variation in pathogenicity and parasitism within the natural population of *T. maxima*. Objective: To compare pathogenicity and parasitism of different isolates of *T. maxima* on different white pines.

b. Other fungi

(1) 2303-94. Fungal inhabitants of western white pine blister rust cankers. Objective: To investigate the fungi, except *T. maxima*, associated with blister rust cankers, and to determine what effect these fungi may have on canker development. The specific objectives are (1) to collect, identify, isolate, and grow in pure culture the fungi found associated with western white pine blister rust cankers and, (2) to evaluate these fungi as potential biological control agents.

(2) 2303-____. Fungal inhabitants of blister rust cankers on the white pines of the central and eastern European countries. Objective: To collect, identify, isolate, and grow in pure culture those fungi found associated with blister rust cankers on white pine species of central and eastern Europe.

c. Insects

(1) 2303 and 2202-____. Identification of insects associated with white pine blister rust cankers and their relationship to such cankers. Objective: To identify the insects commonly associated with blister rust cankers and assess their effect on the canker.

2. Evaluation of Natural Inactivation

a. Impact of natural inactivation

(1) 2303-91____. Levels and trends of natural inactivation. Objective: To determine annually the level of apparent natural inactivation of the canker population in chemically untreated young white pine stands and to define the trend of this inactivation over time.

(2) 2303-92. Persistence of natural inactivation. Objective: For cankers judged inactive at any time, to determine whether they remain inactive or subsequently become active. To establish probabilities for cankers remaining inactive or becoming active.

(3) 2303-____. Significance of natural canker inactivation. Objective: From a given population of infected trees, to predict the number that can be saved from death by blister rust through natural control of cankers. Using information from studies 91 and 92 above, we will assess the capability of natural control to significantly reduce losses from blister rust and, thereby, determine the need for managers to impose supplemental chemical or biological control measures.

b. 2303-____. Influence of site factors on natural inactivation. Objective: To assess the effects of physiographic, edaphic, and other environmental factors on the levels of natural inactivation.

c. Remote sensing for canker and disease evaluatior

(1) PSW-2204-____. Design, development, and testing equipment for remote sensing application. Objective: Based on temperature differences in healthy and rust infected tissues, to design and/or acquire equipment capable of measuring temperature in the indicated temperature regime.

(2) PSW-2204-____. Application of remote sensing equipment and techniques to canker viability determinations. Objective: To determine whether remote sensing equipment designed for field use can reliably measure temperature differences reflective of variations in canker viability.

(3) PSW-2204-____. Application of remote sensing techniques to evaluation of the blister rust disease in trees and stands. Objective: To evaluate the feasibility of utilizing remote sensing for detecting the disease in trees and stands and of using it as an appraisal both for and of control in stands.

Silvicultural

As we view the opportunities for control of forest diseases, rare indeed is the situation that might not best be handled by some alteration(s) of silvicultural and/or management techniques available to the land manager. In the case of the blister rust disease of western white pine, we cannot yet assure management that the silvicultural approach to control can be converted to a practical tool--essentially that the risk will be tolerable. We can, however, say that concept is sound enough biologically to embark on experimentation to establish its practicability.

Theoretical Projections

Certain assumptions are appropriate to the practicality of applying silvicultural control. In spite of differing viewpoints, these appear valid at the moment; but they must eventually meet management and economic criteria and, thus, have to be tested over time. Our assumptions are:

(1) Rotations must be planned for 50 years or shorter duration. Flexibility in management might permit longer rotations dependent on stand evaluation and projected losses at ages 40 to 50.

(2) Softwood lumber of the quality currently supplied by ponderosa and white pine will be a "demand" product 30 to 100 years hence. Pruned white pine stems to supply part of that demand will be an economic venture.

Considering our above assumptions to be valid, we have illustrated in the following several pages the biological soundness of silvicultural control with a theoretical example, fortified as completely as possible with observed biological facts about the rust (Component I-C). Many medium or high sites, which are planned for reforestation and could be regenerated to white pine or white pine in mixture, can be projected through the following course of reasoning. However, we have used an average 15-year-old stand, representing one of a number of stands regenerated predominantly to white pine over the past 20 years (approximately 32,000 acres), to exemplify our case.

At 15 years of age, the average stand now has about 47 percent of its stems infected, about 89 percent of these will be lethally infected. Stocking (density) control at this age to provide a maximum of 400-500 stems/acre will produce a relatively open stand. Thinning should reduce the infection percentage in residual white pine, but in any case, trees with bole cankers would be removed in thinning operations. Nearly all of the cankers (mostly lethals) should be on branches in the lower 5 to 8 feet (or third) of the crown. Thinning (stocking control) at this age should also assure near optimum growth of the trees at least for the next 10 years and possibly more. All of the sampled stands in our study, 2303-91, near the age of 15 have come through the brush stage and have maintained good terminal growth in recent years, up to 2 feet per year. This type of growth rate can produce trees whose subsequent branch growth reduces the hazard from rust and seems compatible with production concepts of management.

Pruning at this stage would remove the lower third of the crown, most of the infection, and still should not impair growth. It will have accomplished removal of a good share of the crown that retains foliage at the highest infection risk and in which branches with the highest risk (those with short annual growth segments) predominate. Trained crews could spot prune infections in the remaining crown at little extra cost; and, if this first pruning job is conducted along with the thinning (or cleaning), this pruning would likely be the least expensive one in the pruning series.

After thinning and pruning are accomplished in this 15-year-old stand, most infections occurring in the next 10 years should still be restricted to the lower third of the bole length. Whether changes in the microenvironment of the tree induced by thinning and pruning will act in favor of or against the disease is unknown, but, conservatively, both operations should assist us by reducing the number of instances when conditions for infection are favorable. In any case, any new infection in this period should rarely get to the bole because foliage in the most likely target portion of the crown-area will be at the branch terminals, and because canker growth, even on branches with favorable annual growth segment lengths, is not rapid enough in the 10-year period to effect bole entry from distances represented by the foliage component.

The decisions to prune a second and perhaps third time in the 15-year-old example can be based on several factors. If the stand will reach a height to permit pruning to 17 feet within 25 years, only a second pruning may be required, since nearly all infections in this 10-year interval will likely exceed the limits to become bole cankers. But, if height growth fails to produce trees prunable to 17 feet, infection pattern and intensity (from 2303-118) will dictate whether pruning should be deferred past 25 years. Should a third pruning be required to reach 17 feet, the earliest age when this condition is met after the second pruning can be chosen without disease considerations entering the decision.

The principle used in selecting 50 years for the rotation length is inherent in the decisions setting the pruning intervals above. After the 17 feet of pruned height is achieved (which may be as early as 25 to 30 years of age), the risk of loss due to rust increases because the unpruned portions of those crowns not already infected will probably become infected. Crown closure, unless a second thinning is made to maintain an open condition and/or growth rate, may force the infection relatively higher in the crown (above the general 1/3 level). In any case, branches in this portion of the crown will likely persist to age 50, will increase in diameter, and thus provide eventual entry to the bole for many infections that reach the primary branches. While information must be collected on trees grown under a thinning-density environment to ascertain the lethality of this canker population, 20 years after the last pruning seems to be the probable limit in time to carry the stand before the risk of substantial losses from rust becomes an undesirable management risk. As long as stands have not closed completely, loss of portions of the crown at 45 to 50 years may not greatly affect growth. Thus, the decision to extend the rotation beyond 50 could be flexible depending on an appraisal reflecting potential direct (mortality) and indirect losses (growth rate, beetle losses, etc.)

Future Research

It is significant that the foregoing case and principles representing it are not devoid of risks. Among these risks are the unknown infection intensities due to wave years and especially favorable sites for infection, the frequencies of bole infections through stem needles and "short shoots," the frequency of bole infections in the upper crown from normal branch cankers, and our capability of bringing a sufficient number of uninfected and treatable trees through the first 10 years of the rotation. Our proposed research on infection patterns (2303-118) will provide information to appraise these risks with particular emphasis to response under treated (pruned and thinned) situations. As our knowledge increases from studies on canker growth characteristics (I-C-1) and on natural inactivation influences (III-B-2a and -b), we will be better prepared to modify treatments to increase efficacy of silvicultural control.

In addition, certain practices could be followed that should improve the biological soundness of silvicultural control measures. Fertilizing coupled with thinning has increased growth rate and could be employed to increase the effectiveness of branch node lengths in hazard reduction, as well as to shorten the rotation, thus, decreasing the time that trees are exposed to the rust. The same advantage would accrue from genetically controlled strains of white pine relative to growth rate, branch size and number, and other crown factors influencing etiology of the disease. The resistant stock may not have to be as highly resistant as planned or might utilize, for example, more parents whose resistance transmission is reflected in reduced canker growth rates or rust hazard reduction, such as a lower number of needle infections than the average lot or premature shedding of infected needles. Pruning intervals can be shortened and pruning heights could be raised above the 17 feet.

But while all of these plus and minus factors merit consideration, the proof of silvicultural control will be tests conducted on a variety of sites. These tests, coupled with evaluation of the most critical biological factors and several studies directed at appraisal of tree growth response to treatment, form the core of our proposed program for the present. All are directed at developing and demonstrating a treatment program, acceptable to management, to reduce the hazard of blister rust infections. Developing criteria for making decisions on the timing of these operations is an integral part.

Problem Area Outline

As for previous problem areas and for reference in Table 1, proposed studies with their objectives are outlined as follows:

A. Silvicultural

   1. Impact of pruning and thinning

      a. 2303-116. Effectiveness of pruning and thinning treatments for blister rust control. Objective: To determine the effects of pruning to various heights and frequencies and of thinning to different stocking levels on rust intensification and its ensuing hazard to management of western white pine.

      b. Growth responses to treatments

         (1) 1201-___. Lumber quality and volume production. Objective: To determine the effects of the above treatments in 1-a above on volume and quality of lumber produced.

(2) 1201-____. Effect of tree response on annual branch segments and diameters. Objective: Using trees in 1-a above, to determine how tree response affects branch growth segments and diameters and how these influence canker lethality.

(3) 1201-562. Pruning effects on growth of young western white pine, Snyder Creek, 1969. Objectives: To determine the effect of pruning, applied to young western white pine as a blister rust control measure, on the subsequent growth of trees that had been planted at five different spacings.

2. Economic evaluation

a. 4201-____. Cost-benefit analyses. Objective: Using cost, mensurational, and effectiveness data (including data as pertinent from Component I), determine whether pruning and thinning would be profitable control measures in white pine management.

RESEARCH PRIORITY

We have listed the studies, which were discussed under the three research components and their subdivisions in the preceding section (pages 11-68), and have indicated their present order of priority in Table 1. For those studies now underway or planned in the relatively near future, we have shown the starting date and an estimated date of termination; some studies have an indefinite status.

Table 1--Summary of problem analysis showing priorities, starting and termination dates, and cooperation for existing and planned studies, FS-INT-2303.

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation[3]
I. Biology of *Cronartium ribicola* and its hosts					
A. Cultural Parameters	1. Host				
	a. 2303-90. Nutritional and environmental requirements--white pine tissue.	3	1966	Indefinite	
	b. 2303-123. Nutritional and environmental requirements--*Ribes* tissue.	2	1969	1971	
	2. Host-Parasite combination				
	a. 2303-93. Culture of white pine tissues infected with rust	3	1966	1969	
	b. 2303-126. Nutritional and environmental requirements.	2	1969	1974	
	c. 2303- . Establishment of rust in *Ribes* tissue cultures.	2	1970	1972	
	3. Parasite				
	a. 2303-98. Establishment of *C. ribicola* in axenic culture.	3	1966	Indefinite	
	b. 2303-124. Pure culture of *C. ribicola* from its vegetative stages.	1	1969	Indefinite	

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[3] Excludes project funding provided by Pest Control, W.O., and the use of various experimental areas.

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
I. A. Cultural Parameters					
	c. 2303-127. Isolation and identification of specific factors ... of *C. ribicola*.	1	1970	1975	
	d. 2303-___. Nutritional and environmental requirements of *C. ribicola* in culture.	3	Indefinite	Indefinite	
B. Physiology of disease and component organisms					
	1. Identification of systems				
	a. Protein extraction, function, and kinetics				
	(1) 2303-101. Extraction of protein from white pine bark.	1	1967	1970	
	(2) 2303-109. Pectic enzymes associated with rust.	1	1968	1969	
	(3) 2303-___. Identification of enzyme function in blister rust disease and role in sustaining *C. ribicola*.	3	Indefinite	Indefinite	
	(4) 2303-___. Kinetics of enzyme reactions involved in disease.	3	Indefinite	Indefinite	

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
I.					
B. Physiology of disease and component organisms	b. Carbohydrate utilization, function, and translocation				
	(1) 2303-128. Influence of blister rust on the seasonal distribution of carbohydrates ... western white pine.	1	1970	1974	
	(2) 2303-____. Role of phenolics in rust metabolism.	2	1971	Indefinite	
	(3) 2303-____. Flavonoids--synthesis and involvement in host and fungus metabolism.	1	1970	Indefinite	
	c. Mineral extraction, function, and kinetics				
	(1) 2303-____. Concentration of inorganic solutes as influenced by rust.	2	Indefinite	Indefinite	FS-INT-2302
	2. Translocation and accumulation				
	a. 2303-107. Translocation of dyes in infected white pine.	2	1968	1971	
	3. Energy relations				
	a. 2303-119. Temperature regimes in healthy and infected white pine tissues.	1	1969	1972	

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
I. B. Physiology of disease and component organisms					
	b. 2303. Breaking dormancy in white pine. (Supplemental to 2302-121).	3	1970	1972	FS-INT-1401 and -2302
C. Epidemiology and etiology of the disease					
	1. Canker characteristics				
	a. 2303-___. Canker growth rates.	3	Indefinite	Indefinite	
	b. 2303-___. Seasonal and yearly expression of canker activity.	2	1970	Indefinite	
	c. 2303-___. Lethality of branch cankers.	3	Indefinite	Indefinite	
	2. Infection patterns				
	a. 2303-118. Distribution of infection in crowns.	1	1969	1974	
	b. 2303-___. Effect of microenvironment.	3	Indefinite	Indefinite	
D. Life Cycle	1. Adaptive capacity to substrates				
	a. 2303-___. Nutritional specificity of isolates.	1	Indefinite	Indefinite	
	b. 2303-___. Pathogenicity of isolates.	3	Indefinite	Indefinite	

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
I. D. Life Cycle	c. 2303-115. Vegetative propagation procedures for *C. ribicola in vivo*.	1	1969	1972	
	2. Sporulation				
	a. 2303-113. Factors affecting formation of *C. ribicola* aeciospores *in vitro*.	2	1969	1972	
	b. 2303-114. Stimulation of aecia production by *C. ribicola in vivo*.	3	1969	1972	
	c. 2303-102. Chromatographic analysis of pycnial fluid.	1	1967	1971	
	d. 2302-____. Sexual nature and genetic variation *in vivo*.				FS-INT-2303
	3. 2303-____. Cytology and ontogeny of *C. ribicola*.	2	1970	1974	
II. Integration of biological information					
	A. 2303-104. Information service for chemical and/or biological control studies on rust.	1	1967	1972	W.S.U.

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
III. Application of biological information in control measures					
A. Chemical	1. 2303-____. Selection of chemical candidates.	2	1970	Indefinite	
	2. 2303-95. Development and implementation of assessment program for screening.	1	1966	Indefinite	
	3. Pilot testing of promising chemicals.	3	Indefinite	Indefinite	S&P (R-1)
B. Biological					
	1. Evaluation of biological agents				
	a. *Tuberculina maxima*				
	(1) Biology				
	(a) 2303-83. Nutritional requirements.	1	1965	1970	
	(b) 2303-84. Ecology.	1	1965	1971	
	(c) 2303-78. Incubation period.	1	1964	1969	
	(d) 2303-____. Host range.	3	Indefinite	Indefinite	
	(e) 2303-____. Spread.	3	Indefinite	Indefinite	

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
III. B. Biological	(2) Effectiveness				
	(a) 2303-75. Effects on rust cankers.	1	1964	1975	
	(b) 2303-77. Time required for *T. maxima* to inactivate infections.	1	1964	1975	
	(c) 2303-112. Mode of action of *T. maxima* in inactivation of rust cankers.	1	1969	1974	
	(d) 2303-____. Natural variations in pathogenicity and parasitism.	2	1971	Indefinite	
	b. Other fungi				
	(1) 2303-94. Fungal inhabitants of rust cankers.	1	1966	1970	W.S.U.
	(2) 2303-____. Fungal inhabitants of rust cankers on white pines of central and eastern European countries.	1	1970	1972	
	c. Insects				
	(1) 2303 and 2202-____. Identification and role or canker insects.	3	1970	1975	FS-INT-2202

Problem components and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
III.					
B. Biological	2. Evaluation of natural inactivation				
	a. Impact of natural inactivation				
	(1) 2303-91. Levels and trends.	1	1966	Indefinite	
	(2) 2303-92. Persistence.	1	1966	Indefinite	
	(3) 2303-____. Significance.	2	Indefinite	Indefinite	
	b. 2303-____. Influence of site factors on inactivation.	2	1971	Indefinite	FS-INT-1201
	c. Remote sensing for canker and disease evaluation				
	(1) PSW-2204-____. Equipment design, development, and testing.	1	Indefinite	Indefinite	FS-INT-2303
	(2) PSW-2204-____. Application to canker viability determinations.	1	Indefinite	Indefinite	FS-INT-2303
	(3) PSW-2204-____. Application to evaluation of the disease in trees and stands.	3	Indefinite	Indefinite	FS-INT-2303
C. Silvicultural	1. Impact of pruning and thinning				
	a. 2303-116. Effectiveness of pruning and thinning treatments for rust control.	1	1969	Indefinite (R-1),	TM, S&P (R-1), FS-INT-1201 PFI
	b. Growth responses to treatments				

Problem component and subdivisions	Problem areas and related studies	Priority	Starting date	Estimated termination date	Cooperation
III.					
C. Silvicultural	(1) 1201-____. Lumber quality and volume production.	3	Indefinite	Indefinite	FS-INT-4301 ?
	(2) 1201-____. Effect of tree response on branch node length and diameter.	3	1975	1984	FS-INT-2303
	(3) 1201-567. Pruning effects on growth of young western white pine.	1	1969	Indefinite	FS-INT-2303
2.	Economic evaluation	3	Indefinite	Indefinite	FS-INT-1201 and -2303
	a. 4201-____. Cost benefit analyses.				

COOPERATION

Research Work Units FS-INT-2302 and -2303 have a rather unique status in the Forest Service since they have an active review committee composed of personnel from certain Washington Office research and administration divisions, Region 1--Timber Management and Pest Control, and the Intermountain Station. Chairmaned by the Chief of Pest Control in the W.O., the Steering Committee was started in 1966 and functions by review and in decisions relative to the program, coordination, and funding of research and development on the blister rust disease.

A most significant aspect of our current cooperation is the funding provided INT-2303 through Pest Control of the Washington Office. More than one-half of the current budget comes from this source, which also provides additional funding toward the development of the information retrieval service (2303-104). The second of our major cooperators is Washington State University which not only is involved in two studies (2303-94 and -104) but also provides equipment sharing, computer services, professional contact and advice, and a supply center for laboratory equipment and materials. Our third major cooperation involves the national forests and Regional Office in Region 1 and Potlatch Forests, Inc., who have greatly facilitated our research by the provision of land for experimental use, funds and/or personnel to complete installation of field experiments, and technical assistance in the field.

Since 1966, Research Work Units 2302 and 2303 have coordinated their activities; but, as our respective research programs emphasize studies toward an understanding of the physiology of the rust disease, cooperation and coordination obviously become clear mandates. When we extend our research into silvicultural manipulation of stands for disease control, cooperation between Research Work Unit FS-INT-1201 and our project will become quite active. Also, as we have done in the past, we will utilize our cooperative arrangements with Washington State University and the University of Idaho to complete studies in our program that might otherwise be deferred due to lack of facilities, manpower, etc.

EQUIPMENT, FACILITIES, MANPOWER, AND FINANCES

At our present state of knowledge, our current professional staff is capable of directing research envisioned in this analysis. As our knowledge base expands, we might increase this staff, but within the next three years, we critically need to increase only our support staff of trained, capable technicians. Two permanent technicians per scientist would be an acceptable goal within two years with a goal in four years of the equivalent of three permanents.

If adequate laboratory space were available now, the above technical support goals could be shortened and would increase our production considerably. Even though available work space is now somewhat crowded, the minimum goal can be accommodated. Until such time that the proposed second phase of the Forestry Sciences Laboratory and the greenhouse-headhouse facilities at Moscow are completed, no other increase in staffing (professional or technical) could be handled. There is some doubt that, even in the new phase, an increase in professional staffing could be accommodated because no laboratory space (other than the multipurpose radiology lab) is earmarked for this project. Five scientists with two to three technicians each will severely test the capacity of the two laboratories now used.

With one major exception, our equipment is adequate to meet most anticipated needs other than usual minor items. That exception is the lack of radiotracer equipment and the facility to use it in. Such a facility is provided for in the new phase of Laboratory construction, but it will have to be equipped. Items have already been catalogued so that, when the time is appropriate and the funds are available, purchase can occur immediately. In the interim, many studies that might otherwise have been rated higher in priority will be delayed. Certain moderate or low priority studies were not identified because of lack of radiotracer facilities. We may conduct some very high priority work in this area at the University of Idaho or Washington State University but only on a piecemeal basis that would not interrupt their programs.

Financing is always a critical subject of concern. Although Rasmussen's memorandum of April 1, 1966, to the Regional Forester (R-1) and the Director (INT) outlined a likely financial base in the Memorandum of Understanding, it did not spell out time schedules for financing research and development. Not only do we need to increase our technical support but we need to keep our budget base solvent, i.e., capable of absorbing annual increases in costs of doing business and not affect our productive effort. Therefore, on the basis of an average cost of $10,500 to support one technician (based on our present staff in terms of productive capacity of the scientist supervisors) and of annual increases in operating costs of four percent, we would need by fiscal year 1973 a total increase of about $117,000 at the project level above the 1969 base of $212,728. This figure, which is well within the base set by the Memorandum of Understanding, incorporates a release of presently committed funds for the retrieval system (2303-104) by F.Y. 1973, but does not include a cost of between $9,000 and $10,000 for radiotracer equipment in the year that the radiology laboratory is provided.

REFERENCES

Allen, P. J. 1953. Toxins and tissue respiration. Phytopathology 43: 221-229.

Anderson, J. W. 1968. Extraction of enzymes and subcellular organelles from plant tissues. Phytochemistry 7: 1973-1988.

Arthur, Joseph C. 1929. The Plant Rusts (Uredinales). John Wiley and Sons, Inc., 446 pp.

Barkai-Golan, R. 1959. Tuberculina persicina (Ditm.) Sacc. attacking rust fungi in Israel. Bull. Res. Coun. Israel, Sect. d, 8: 41-46.

Bateman, D. F., and R. L. Millar. 1966. Pectic enzymes in tissue degradation. Ann. Rev. of Phytopathology 4: 119-146.

Bauch, R., and U. Simón. 1957. Kulturversuche mit Rospilzen. Ber. deut. botan. Ges. 20: 145-156.

Bingham, Richard T., and John Ehrlich. 1943. A preliminary field study of the fungi associated with blister rust cankers on western white pine. Univ. of Idaho, School of Forestry Report, 30 pp. (typewritten.)

Bingham, R. T., R. J. Olson, W. A. Becker, and M. A. Marsden. 1969. Breeding blister rust resistant western white pine. V. Estimates of heritability, combining ability, and genetic advance based on tester matings. Silvae Genetica 18: 28-38.

Brian, P. W. 1954. The use of antibiotics for the control of plant diseases caused by bacteria and fungi. J. Appl. Bacteriol. 17: 142-151

Brooks, F. T. 1928. Plant Diseases. Oxford Univ. Press, London. vi + 386 pp.

Byrde, R. J., S. N. Crowdy, and D. Woodcock. 1953. Studies on systemic fungicides. III. The activity of certain chlorine-substituted β-napthols and napthyloxy-n-aliphatic carboxylic acids as systemic fungicides. Ann. Appl. Biol. 40: 152-165.

Calvert, W. W., and L. G. Brace. 1969. Pruning and sawing eastern white pines. Dept. of Fisheries and Forestry. Can. Forestry Serv. Publ. No. 1262, 22 pp.

Carlson, C. E., and H. V. Toko. 1968. Preliminary report on white pine blister rust incidence survey--1966 and 1967. U.S.D.A. Forest Service, Div. of State and Private Forestry, Region 1. 3 pp. and 4 figs.

Chapman, C. H. 1934. The relation of needle infection location to the incubation period of blister rust. Western Blister Rust Newsletter (U.S.D.A., B.E.P.Q., Spokane, Wash., mimeo.) 9: 39-42, June.

Colley, R. H. 1918. Parasitism, morphology, and cytology of *Cronartium ribicola*. Jour. Agri. Res. 15: 619-660.

Conn, George Keith Thurburn, and D. G. Avery. 1960. Infrared Methods: Principles and Application. Academic Press. New York. 203 pp.

Crowdy, S. H. 1959. Uptake and translocation of organic chemicals by higher plants. pp. 232-238. IN: Plant Pathology, Problems and Progress: 1908-1958. Univ. of Wisconsin Press.

Crowdy, S. H., and R. L. Wain. 1951. Studies on systemic fungicides. I. Fungicidal properties of the aryloxyalkylcarboxylic acids. Ann. Appl. Biol. 38: 318-333.

Crowdy, S. H., and D. Pramer. 1955. Movement of antibiotics in higher plants. Chem. Ind. pp. 160-162.

Cutter, V. M., Jr. 1951. The isolation of plant rust upon artificial media and some speculations on the metabolism of obligate plant parasites. Trans. N.Y. Acad. Sci. 14: 103-105.

______________. 1952. Observations on the growth of *Uromyces caladii* in tissue cultures of *Arisaema triphyllum*. Phytopathology 42: 479. (Abstr.)

______________. 1959. Studies on the isolation and growth of plant rusts on host tissue cultures and upon synthetic media. I. *Gymnosporangium*. Mycologia 51: 248-295.

______________. 1960. Studies on the isolation and growth of plant rusts in host tissue cultures and upon synthetic media. II. *Uromyces ari-briphylis*. Mycologia 51: 248-295.

Daubenmire, R. F., and Jean B. Daubenmire. 1968. Forest vegetation of eastern Washington and northern Idaho. Wash. Agr. Exp. Sta. Tech. Bull. 60, 104 pp., illus.

Deacon, G. E. 1939. Notes on a parasitic fungus attacking rose rust. Rose Ann. 1939: 136.

Dickson, J. G. 1959. Chemical control of cereal rusts. Botan. Rev. 25: 486-513.

Dimond, A. E. 1958. Plant Chemotherapy, Problems and Progress: 1908-1958. Univ. of Wisconsin Press. 1959.

__________. 1965. Natural models of chemotherapy. Adv. in Pest Control Res. 6: 127-169.

Dimond, A. E., and D. Davis. 1952. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agri. Expt. Sta., New Haven, Bull. 557.

Dimond, A. E., and J. G. Horsfall. 1959. Plant chemotherapy. Ann. Rev. of Plant Physiology 1: 257-273.

Durbin, R. D. 1967. Obligate parasites: Effect on the movement of solutes and water. pp. 80-99. IN: The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction. Eds. C. J. Mirocha and I. Uritani. The American Phytopathological Society, Inc., St. Paul, Minnesota. X + 372.

Fawcett, C. H., D. M. Spencer, and R. L. Wain. 1955. Investigations on fungicides. I. Fungicidal and systemic fungicidal activity in certain aryloxyalkanecarboxylic acids. Ann. Appl. Biol. 42: 300-317.

Fischer, G. W., and C. S. Holton. 1957. Biology and Control of the Smut Fungi. The Ronald Press Co., New York, New York.

Flood, A. E., and D. S. Kirkham. 1960. The effect of some phenolic compounds on the growth and sporulation of two _Venturia_ species. pp. 81-85. IN: Phenolics in Plants in Health and Disease. Ed. J. B. Pridham. Pergamon Press, New York IX + 131.

Gautheret, R. J. 1942. Manual technique de culture des tissus vegetaux. Masson et. Cie, Paris.

Gobi, C. von. 1885. Über den _Tubercularia persicina_, Ditm. genannten Pilz. Memoires de L'Academie Imperiales des Sciences de St.-Petersbourg, VII Serie 32: 1-26.

Goodding, L. N. 1932. Notes on biological control of blister rust. Western Blister Rust News Letter 7: 51-53.

Greenidge, K. N. H. 1955a. Observations on the movement of moisture in large woody stems. Can. J. Bot. 33: 202-221.

______________. 1955b. Studies in the physiology of forest trees. III. The effect of drastic interruptions of conducting tissues on moisture movement. Am. J. Botany 42: 582-587.

______________. 1957. Ascent of sap. Ann. Rev. Plant Physiol. 8: 237-256.

Hackforth, Henry L. 1960. Infrared Radiation. McGraw-Hill, New York. 303 pp.

Hadwiger, L. A., and M. E. Schwochau. 1969. Host resistance--an induction hypothesis. Phytopathology: 11-12. (Abstr.)

Harborne, J. B. 1964. Biochemistry of Phenolic Compounds. Academic Press, New York. X + 618.

Harvey, A. E. 1967a. Axenic culture of western white pine cambial explants infected with blister rust. Phytopathology 57: 1005. (Abstr.)

__________. 1967b. Tissue culture of *Pinus monticola* Dougl. on a chemically defined medium. Can. J. Bot. 45: 1783-1787.

Harvey, A. E., and J. L. Grasham. 1969. Growth of the rust fungus *Cronartium ribicola* in tissue cultures of *Pinus monticola*. Can. J. Bot. 47: 663-666.

Harvey, A. E., and J. L. Grasham. 1970a. Growth of *Cronartium ribicola* in the absence of physical contact with its host. Can. J. Bot. 48: 71-73.

Harvey, A. E., and J. L. Grasham. 1970b. Survival of *Cronartium ribicola* on a medium containing host tissue culture diffusates. Mycopathologia et Mycologia Applicata. (In press.)

Harvey, A. E., and J. Y. Woo. 1969. Some cytological characteristics of *Cronartium ribicola* in tissue cultures of *Pinus monticola*. Phytopathology 59: 12. (Abstr.)

Hirt, Roy R. 1964. *Cronartium ribicola*: Its growth and reproduction in the tissues of eastern white pine. State U. Coll. of Forestry, Syracuse U. Tech. Publ. No. 86, 30 pp + 14 plates.

Horsfall, J. G. 1956. Principles of Fungicidal Action. Chronica Botanica, Waltham, Mass. 280 pp.

Horsfall, J. G., and A. E. Dimond. 1951. Plant chemotherapy. Ann. Rev. of Microbiology 5: 209-222.

Hotson, H. H. 1953. The growth of rust in tissue culture. Phytopathology 43: 360-363.

Hotson, H. H., and V. M. Cutter, Jr. 1951. The isolation of Gymnosporangium juniperi-virginianae Schw. upon artificial media. U.S. Nat. Acad. Sci. Proc. 37: 400-403.

Hubert, E. E. 1932. Biological control by means of the purple mold. Western Blister Rust News Letter 7: 96-98.

___________. 1935. Observations on Tuberculina maxima, a parasite of Cronartium ribicola. Phytopathology 25: 253-261.

Hulea, A. 1939. Contributions a la connaissance des champignons commensaux des Uredinees. (Contributions to the knowledge of fungi commensal with the Uredineae). Bull. Sect. Sci. Acad. Council 22: 1-19.

Hulme, A. C., and J. D. Jones. 1963. Tannin inhibition of plant mitochondria. pp. 97-120. IN: Enzyme Chemistry of Phenolic Compounds. Ed. J. B. Pridham. The MacMillan Co., New York. IX + 142.

Keener, P. D. 1954. Cladosporium aecidiicola Thuem. and Tuberculina persicina (Ditm.) Sacc. associated with Puccinia conspicua (Arth.) Mains on Helenium hoopesii A. Gray in Arizona. Plant Dis. Reptr. 38: 690-694.

Kendrick, E. L. 1957. The production of teliospores of Tilletia caries in culture. Phytopathology 47: 674-676.

Kimmey, J. W. 1969. Inactivation of lethal-type blister rust cankers on western white pine. J. of For. 67: 296-299.

Kirulis, A. 1942. Die mikroskopischen Pilze als naturliche Feinde der Pflanzenkrankheiten in Lettland. (The microscopic fungi as natural enemies of plant disease in Latvia.) Orb. landu. Akad. Mitan 1: 479-536.

Koenigs, J. W. 1968. Culturing the white pine blister rust fungus in callus of western white pine. Phytopathology 58: 46-48.

Kramer, Paul J., and Theodore T. Kozlowski. 1960. Physiology of Trees. pp. 214-215. McGraw-Hill Book Co., Inc. 642 pp.

Leaphart, C. D., and E. F. Wicker. 1968. The ineffectiveness of cycloheximide and phytoactin as chemical controls of the blister rust disease. Plant Dis. Reptr. 52: 6-10.

Lechmere, E. 1914. Tuberculina maxima Rost. Einparasit duf dem Blasenrost der Weymouthskiefer. (A parasite of white pine blister rust.) Naturw. Ztsch. Forst-u Landu. 12: 491-498.

Lind, J. 1913. Danish Fungi as Represented in the Herbarium of E. Rostrop. 648 pp. Ctyldenolske boghandel, Copenhagen.

Livingston, J. E., and M. T. Hilborn. 1959. The control of plant diseases by chemotherapy. Econ. Botany 13: 3-29.

Loomis, W. D., and J. Battaile. 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry 5: 423-438.

Maheshware, R., A. C. Hildebrandt, and P. J. Allen. 1967. Factors affecting the growth of rust fungi on host tissue culture. Botanical Gazette 128: 153-159.

Martin, N. E. 1967. Histochemical analysis of the blister rust fungus, Cronartium ribicola. Phytopathology 57: 820-821. (Abstr.)

______________. 1968. Concentration of solutes in blister rust infections of western white pine. Phytopathology 58: 1059. (Abstr.)

Matthews, D. N., and S. B. Hutchison. 1948. Development of a blister rust control policy for the National Forests in the Inland Empire. U.S.F.S. Nor. Rocky Mtn. Sta., Station Paper 16, 116 pp.

Mielke, J. L. 1933. Tuberculina maxima in western North America. Phytopathology 23: 299-305.

Mitchell, Russel G. 1967. Translocation of dye in grand and subalpine firs infested by the balsam wooly aphid. Pacific Northwest Forest and Range Expt. Sta., U.S. Forest Serv. Res. Note PNW-46. 17 pp.

Morel, G. 1948. Recherches sur la culture associeé de parasites obligatoires et de tissus végétaux. Ann. Epiphyts. N.S. 14: 123-234.

Moss, V. D., and C. A. Wellner. 1953. Aiding blister rust control by silvicultural measures in the western white pine type. U.S. Dept. Agri. Circ. 919: 1-32.

Neilands, J. B., and P. K. Stumpf. 1958. Outlines of Enzyme Chemistry. John Wiley and Sons, Inc., New York. 411 pp.

Nozzolillo, C., and J. H. Craigie. 1960. Growth of the rust fungus Puccinia helianthi on tissue cultures of its host. Can. J. Bot. 38: 227-233.

Oort, A. J. P., and J. Dekker. 1960. Experiments with rimocidin and pimaricin, two fungicidal antibiotics with systemic action. Proc. Fifth International Congress, Crop Protection 16: 1565-1567.

Oort, A. J. P., and O. M. van Andel. 1960. Aspects of chemotherapy. Mededel Landbouwhogeschool en Opzoekingseta Staat Gent. 24: 981-992.

Patton, R. F. 1962. Inoculation with Cronartium ribicola by bark-patch grafting. Phytopathology 52: 1149-1153.

Peterson, R. S., and F. F. Jewell. 1968. Status of American stem rusts of pine. Ann. Rev. of Phytopathology 6: 23-40.

Pierson, R. K. 1933. Fusion of pycniospores with filamentous hyphae in the pycnium of white pine blister rust. Nature 131: 728-729.

Posey, G. B., and G. F. Gravatt. 1917. Unpublished manuscript "Field studies on the white pine blister rust at Kittery Point, Maine" as reported by Spaulding 1922.

Pridham, J. B. 1960. The formation and possible function of phenolic glycosides. pp. 9-15. IN: Phenolics in Plants in Health and Disease. Ed. J. B. Pridham. Pergamon Press, New York. IX + 131.

Rayss, T. 1943. Contribution a l'etude des Deuteromycetes de Palestine. (Material for the study of the Deuteromycetes of Palestine.) Palestine Jour. Bot. Jour. Ser. 3: 22-51.

Rhoads, A. S. 1920. Studies on the rate of growth and behavior of the blister rust on white pine in 1918. Phytopathology 10: 524-526.

Rohmeder, E. 1931. Anbäuflache und Gefährdungen der Strobe im bayerischen Staatswald. (Area white pine in the Bavarian State Forest and risks to which it is exposed.) Forstwissensch. Centralbl. 53: 325-329.

Rossetti, V. R., and G. Morel. 1958. Le developpment due *Puccinia antirrhini* sur tissus du Muflier cultures *in vitro*. Compt. rend. 247: 1893-1895.

Rostrup, E. 1890. Ustilagineae, Daniae. Festskrift Bot. Forening i Kjbenhaon, April 1890: 117-168. Copenhagen.

Rowell, J. B. 1968. Chemical control of the cereal rusts. Ann. Rev. of Phytopathology 6: 243-262.

Shaw, M. 1963. The physiology and host-parasite relations of the rusts. Ann. Rev. Phytopathology 1: 259.

———. 1964. The physiology of rust urediospores. Phytopathology Z. 50: 159-180.

Sijpesteijn, A. K. 1961. New developments in the systemic combat of fungal diseases of plants. T. Jdschr. 67(1961): 11-20.

Slipp, A. W. 1949. Canker development following artificial inoculation. University of Idaho, School of Forestry, Progress Report on the 1945-46 biennium, 138 pp.

Snell, W. H. 1929a. Some observations upon the white pine blister rust in New York. Phytopathology 19: 269-283.

———. 1929b. *Dasyscypha agassizii* on *Pinus strobus*. Mycologia 21: 235-242.

Spaulding, Perley. 1929. White-pine blister rust: A comparison of European with North American conditions. U.S. Depart. Agri. Tech. Bul. 87, 58 pp.

Stoddard, E. M. 1951. Control of strawberry stele by chemotherapy. Phytopathology 22: 858.

———. 1957. A Fusarium rot of geraniums and its control. Plant Dis. Reptr. 41: 536.

Suzuki, N. 1965. Histochemistry of foliage diseases. Ann. Rev. of Plant Pathology 3: 265-286.

Tani, T. 1967. The relation of soft rot caused by pathogenic fungi to pectic enzyme production by the host. pp. 40-57, IN: The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction. Ed: C. J. Mirocha and I. Uritani. The American Phytopathological Soc., Inc., St. Paul, Minn. X + 372.

Thirumalachar, M. J. 1941. *Tuberculina* on *Uromyces hobsoni* Viz. Jour. Indian Bot. Soc. 20: 107-110.

Trione, E. J. 1963. Isolation and *in vitro* culture of the wheat bunt fungi *Tilletia caries* and *T. controversa*. Phytopathology 53: 592-596.

Tubeuf, C. Von. 1901. Über *Tuberculina maxima* einen Parasiten des Weymouthskiefern-Blasenrostes. (About *T. maxima*, a parasite of white pine blister rust.) Arb. K. Biol. Anst. Landu. Forstw. 2: 169-173.

___________. 1914a. Biologische Bekämpfung von Pilzkrankheiten der Pflanzen. Naturw. Ztschr. Forst. u. Landw. 12: 11-19.

___________. 1914b. Neure Versuche und Beobachtungen über den Blasenrost der Weymouthskiefer. Naturw. Ztschr. Forst. u. Landw. 12: 484-491.

___________. 1930. Biologische Bekampfung des Blasenrostes der Weymouthskiefer. (Biological control of white pine blister rust.) Ztschr. Pflkrankh. 40: 177-181.

___________. 1936. Verlauf und Erfolg der Erforschung der Blasenrostkrankheit der Strobe von 1887 bis 1936. (The progress and outcome of research on blister rust disease of *P. strobus* from 1887 to 1936.) Ztschr. Pflkrankh. 46: 49-103 and 113-171.

Turel, F. L. M., and G. A. Ledingham. 1957. Production of aerial mycelium and urediospores by *Melampsora lini* (Pers.) Lev. on flax leaves in tissue culture. Can. J. Microbiol. 3: 813-819.

Van der Kerk, G. J. M. 1956. The present state of fungicide research. Mededel. Landbouwhogeschool Gent. 21: 305-339.

Vité, J. P. 1959. Observations on the movement of injected dyes in *Pinus ponderosa* and *Abies concolor*. Contrib. Boyce Thompson Inst. 20: 7-26.

Walkinshaw, C. H., F. F. Jewell, and N. M. Walker. 1965. Callus culture of fusiform rust infected slash pine. Plant Dis. Reptr. 49: 616-618.

Wear, John F. 1966. The development of spectro-signature indicators of root disease on large forest areas. Ann. Prog. Report. U.S.D.A., Forest Serv. Pacific Southwest Forest and Range Experiment Station.

Weindling, R., H. Katynelson, and H. P. Beale. 1950. Antibioses in relation to plant diseases. Ann. Rev. Microbiology 4: 247-260.

Weir, J. R., and E. E. Hubert. 1917. Pycnial stages of important forest tree rusts. Phytopathology 7: 135-139.

White, P. R. 1943. A Handbook of Plant Tissue Culture. Jaques Cattel Press, Lancaster, Pa.

Wicker, Ed F. 1968. Toxic effects of cycloheximide and phytoactin to _Tuberculina_ _maxima_. Phytoprotection 49: 91-98.

Wicker, E. F., and J. W. Kimmey. 1967. Mode and time of infection of western white pine blister rust cankers by _Tuberculina_ _maxima_. Phytopathology 57: 1010. (Abstr.)

Wicker, Ed F., and C. G. Shaw. 1968. Fungal parasites of dwarf mistletoes. Mycologia 60: 372-383.

Wicker, E. F., and J. M. Wells. 1968. Overwintering of _Tuberculina_ _maxima_ on white pine blister rust cankers. Phytopathology 58: 391.

Wicker, E. F. and J. Y. Woo. 1969. Differential response of invading _Tuberculina_ _maxima_ to white pine tissues. Phytopathology 59: 16. (Abstr.)

Wikstrom, John H., and Charles A. Wellner. 1961. The opportunity to thin and prune in the northern Rocky Mountain and Intermountain regions. U.S.D.A., Forest Service, Intermountain Forest and Range Experiment Station Research Paper 61: 1-14.

Will, G. M. 1957. Variations in the mineral content of radiata pine needles with age and position in tree crown. New Zealand Forestry Research Notes 11: 699-706.

Williams, A. H. 1963. Enzyme inhibition by phenolic compounds. pp. 87-95. IN: Enzyme Chemistry of Phenolic Compounds. Ed. J. B. Pridham. The MacMillan Co., New York. IX + 142.

Williams, P. G., K. J. Scott, and Joy L. Kuhl. 1966. Vegetative growth of *Puccinia graminis* f. sp. *tritici in vitro*. Phytopathology 57: 1418-1419.

Williams, P. G., K. J. Scott, Joy L. Kuhl, and D. J. Maclean. 1967. Sporulation and pathogenicity of *Puccinia graminis* f. sp. *tritici* grown on an artificial medium. Phytopathology 57: 326-327.

Wilson, M. 1925. A parasite of Weymouth pine blister rust. Nature 115: 661. (Abstr.)

Wollenweber, H. W. 1934. *Fusarium bactridioides* sp. *nov*., associated with *Cronartium*. Science 79: 572.

Wood, R. K. S. 1960. Pectic and cellulolytic enzymes in plant diseases. Ann. Rev. Plant Physiol. 11: 299-322.

Yarwood, C. E. 1953. Heat of respiration of injured and diseased leaves. Phytopathology 43: 675-681.

__________. 1956. Obligate parasitism. Ann. Rev. Plant Physiol. 7: 115-142.

Zaumeyer, W. J. 1956. Improving plant health with antibiotics. Nat'l. Acad. Sci. Natl. Research Council Publ. 397: 171-187.

__________. 1958. Antibiotics in the control of plant diseases. Ann. Rev. of Microbiology 12: 415-440.